acta physiologica latino americana

VOLUMEN IX
Nº 4

1959

ACTA PHYSIOLOGICA LATINOAMERICANA

Editada por la Asociación Ciencia e Investigación

SECRETARIO DE REDACCION
Dr. Manuel R. Malinow

ADMINISTRADORA
Srta. Josefina Yanguas

Obligado 2490, Buenos Aires — Argentina

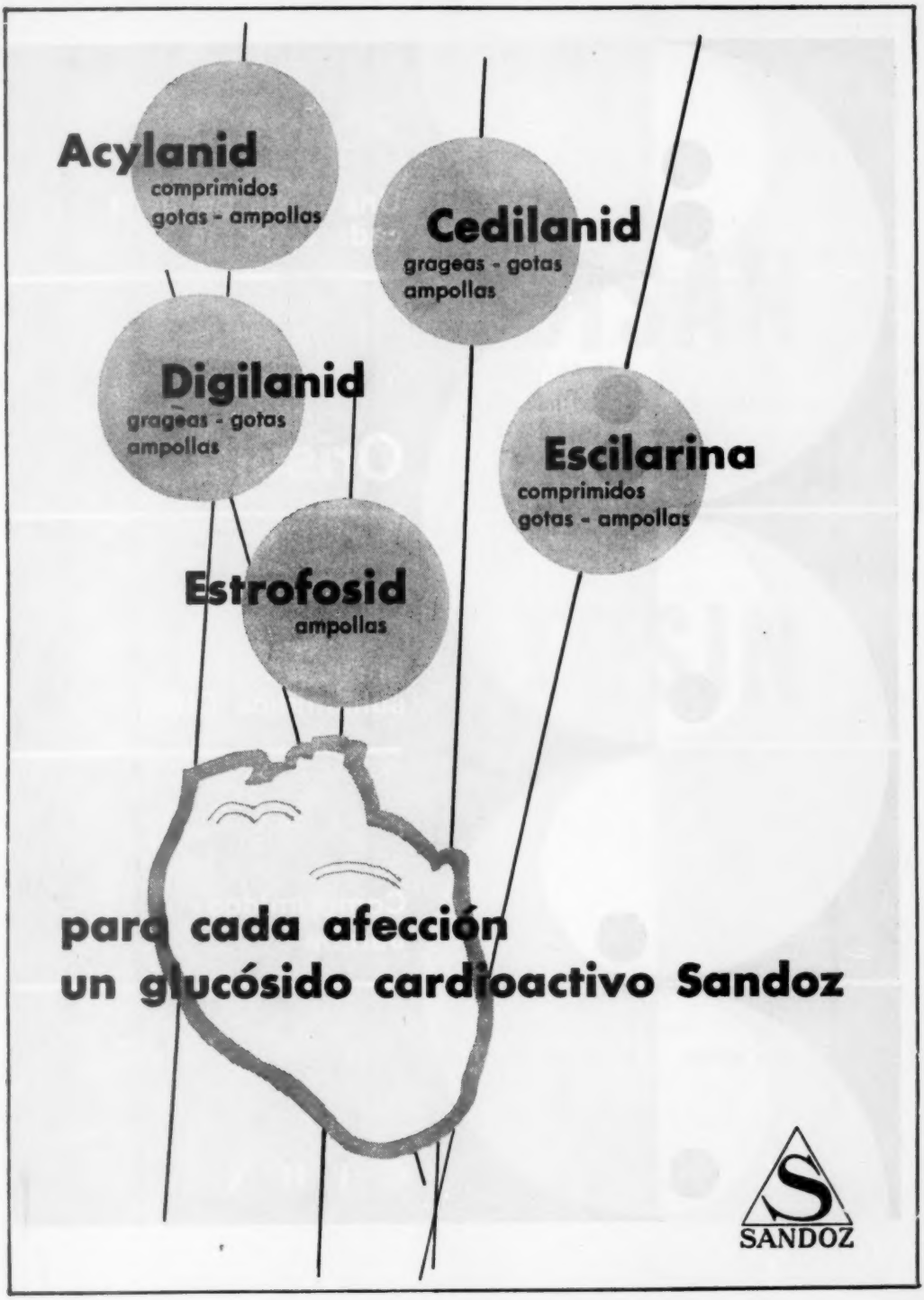
Suscripción

Cada tomo (cuatro números trimestrales) $ 250.— m/n. Exterior: 7.50 dólares

REPRESENTANTES:

BRASIL: Editora Guanabara, Weissmann-Koogan, Ltda.
Rua do Ouvidor, 132, Río de Janeiro.

EE. UU.: Stechert - Hafner Inc.
31 East 10th Street, New York 3, N. Y.



para cada afección
un glucósido cardioactivo Sandoz

SANDOZ

2

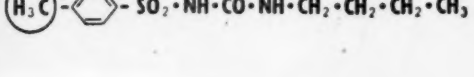
Antidiabéticos orales "BOEHRINGER"

NADISAN

(BZ 55)

ARTOSIN

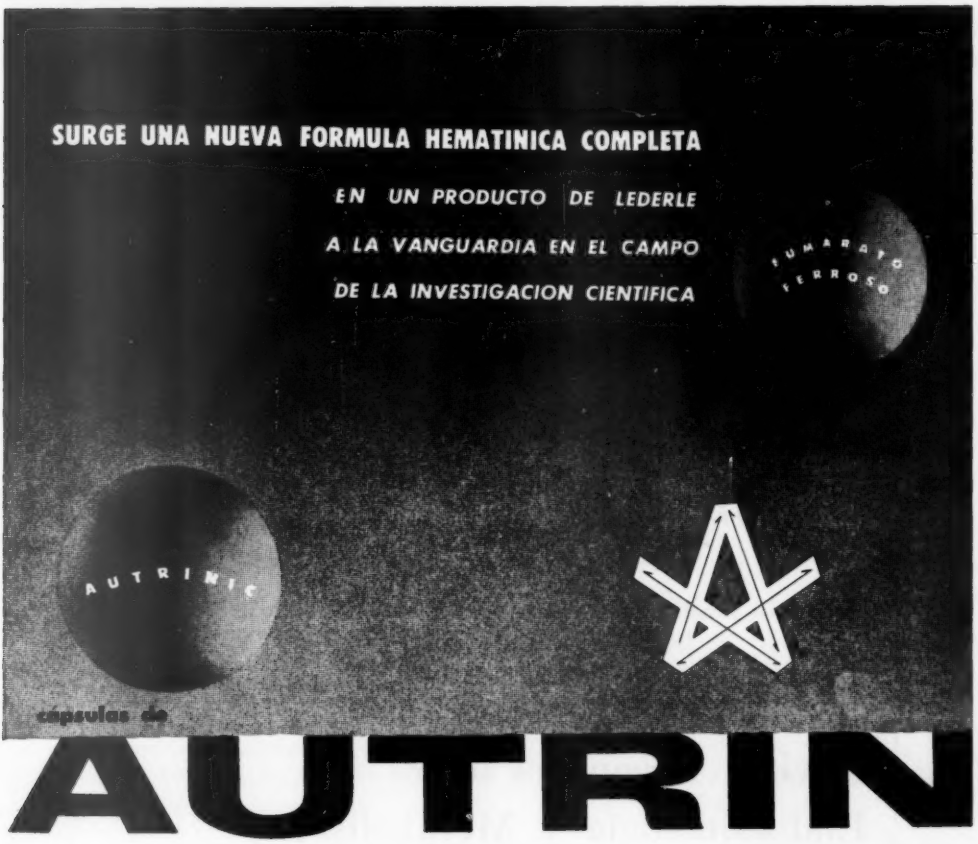
(D 860)



Boehringer Argentina S.A.

ESPECIALIDADES MEDICINALES

S. de BUSTAMANTE 68 BUENOS AIRES



TERAPIA CONSISTENTE EN UNA SOLA CAPSULA DIARIA

HEMATINICO

Cada cápsula de AUTRIN Lederle contiene:

Vitamina B_{12} con Autrinic	2 unidades F.E.U. (oral) (1)
Fumarato de hierro	350 mg
Hierro (como fumarato)	115 mg
Acido ascórbico (C)	150 mg
Acido fólico	2 mg

(1) Potencia determinada antes de agregar los demás ingredientes. El AUTRINIC aumenta la absorción de la vitamina B_{12} en anemia perniciosa y otras dolencias por carencia de vitamina B_{12}

AUTRINIC ®

Factor intrínseco concentrado no inhibitorio... Una exclusiva de *LEDERLE*

FUMARATO FERROSO

Una nueva sal que produce menos irritación gástrica

Lederle

CYANAMID

LABORATORIOS LEDERLE

División de CYANAMID DE ARGENTINA S. A.

CHARCAS 5051/63 - TEL. 72-7031 - BUENOS AIRES

A 6011

ASOCIACION MEDICA ARGENTINA

SOCIEDAD ARGENTINA DE ENDOCRINOLOGIA Y METABOLISMO

SANTA FE 1171 - BUENOS AIRES

PRIMER COLOQUIO SOBRE CORTICOIDES

2, 3 y 4 DE JUNIO DE 1960

BASES DEL CONCURSO

Los trabajos deben versar sobre investigaciones clínicas o experimentales de los corticoides; ser inéditos, de presentación anónima, bajo seudónimo, que se repetirá en un sobre lacrado conteniendo en su interior el título del trabajo y el o los nombres de los autores. Su redacción se ajustará al reglamento de publicaciones de la "Revista Argentina de Endocrinología y Metabolismo", no debiendo hacerse referencias personales que permitan su identificación ni mencionar productos, marcas o nombres comerciales; serán remitidos hasta el 15 de Marzo de 1960, por quintuplicado, al Dr. Grato E. Bur, Secretario General del PRIMER COLOQUIO SOBRE CORTICOIDES, Asociación Médica Argentina, Santa Fe 1171, Buenos Aires.

JURADO:

Dr. ARMANDO TACHELLA COSTA Dr. CARLOS REUSSI

Dr. FELIPE A. DE LA BALZE Dr. ROBERTO MANCINI

PREMIOS: **1º - Medalla de oro y $ 25.000**

2º - Medalla de plata y $ 15.000

3º, 4º y 5º - Diploma y $ 5.000

Especial para trabajo experimental - Medalla de plata y $ 15.000

Los trabajos premiados serán:

a) leídos en las sesiones del Coloquio.
b) publicados por la "Revista Argentina de Endocrinología y Metabolismo".

RELATORES:

Dr. Venancio Deulofeu	Relaciones entre estructura y actividad de los corticoides.
Dr. Alberto Ricca Dr. Mario H. Rigoli	Mineralocorticoides.
Dr. Bernardo Nusimovich	Glucocorticoides.
Dr. Juan J. Staffieri	Corticoides de acción sexual.
Dr. Guido Ruiz Moreno	Corticoides y alergia.
Dr. Raúl Houssay Dr. Samuel Bosch	Corticoides y reumatología.
Dr. Aaron Kaminsky	Corticoides y dermatología.

COMITE ORGANIZADOR:

JOSE MARIA TARRIDA — TESORERO

ARMANDO TACHELLA COSTA — PRESIDENTE

LUCIA AMATO AGUIRRE — SECRETARIA ADMINISTRATIVA

GRATO E. BUR — SECRETARIO GENERAL

ISMAEL E. DE FORTEZA - ISAAC GANOPOL - JORGE SALVANESCHI
VOCALES

patrocinado por SCHERING CORPORATION U. S. A.

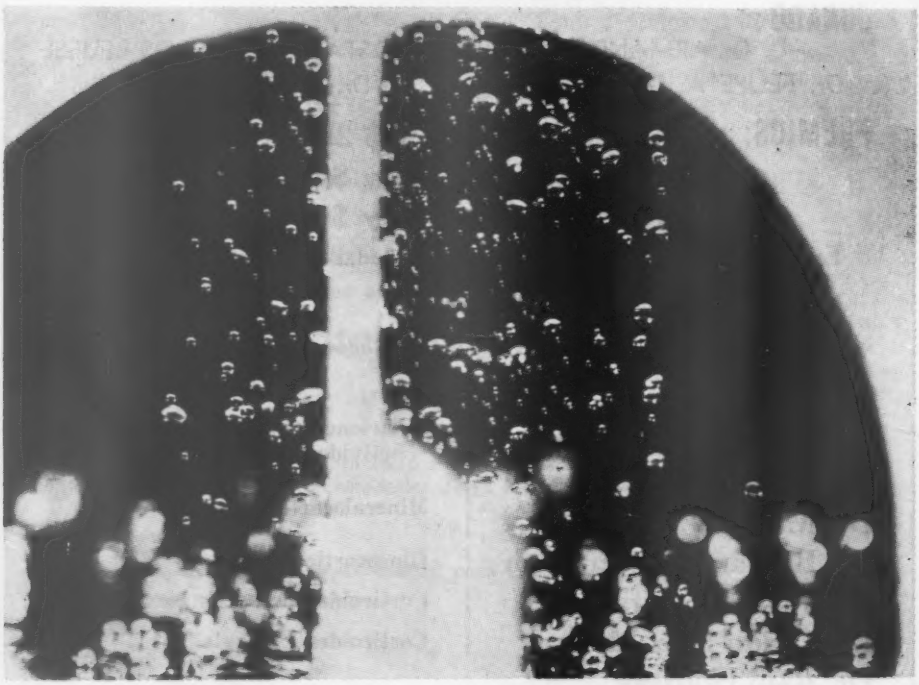
representada por ESSEX (Argentina) S. A. I. C.

en la falta de oxígeno
aguda y crónica

Ampollas,
gotas, perlas

Micorén®

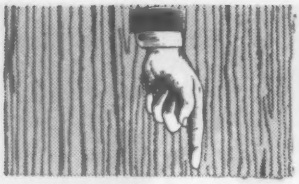
Respirotónico



J. R. Geigy S.A., Basilea (Suiza)
Departamento farmacéutico

Representantes y distribuidores:
Geigy Argentina S.A.
Departamento farmacéutico
Leandro N. Alem 822
Buenos Aires

045 Arg 4



estímulo metabólico directo

La **L-triiodotironina** es probablemente la etapa final de la hormona tiroactiva.

— De acción directa en los procesos metabólicos celulares.
— Constituye un tratamiento seguro y fácilmente controlable de los **estados hipometabólicos** y de la obesidad, dismenorreas, insuficiencia metabólica de la vejez, etc.

Posología: 20 a 60 microgramos repartidos en dos o tres tomas diarias.

Presentación: frasco con 50 comprimidos ranurados (cada comprimido contiene 20 microgramos de L-triiodotironina sódica).

TRI-IODO-TIRONINA GLAXO

GLAXO

LABORATORIOS GLAXO (Argentina) S. A. C. e I.

INFLUENCE OF POSTURAL CHANGES ON AMMONIA EXCRETION BY THE KIDNEY IN AMMONIUM CHLORIDE LOADING

A. C. Taquini, J. D. Fermoso, P. Aramendía, I. de la Riva, B. N. Badano and M. F. Villamil

(Centro de Investigaciones Cardiológicas, Azcuénaga 985, Buenos Aires)

It has been repeatedly demonstrated that changing from recumbency to the standing position determines a sharp decrease in the renal excretion of water, sodium, potassium and chloride ([1-11]). However, data concerning ammonia excretion under these conditions are scarce and contradictory. Judging from a few isolated data from Godyer and Seldin ([8]) it would appear that there is a tendency for ammonia excretion to slightly fall upon standing. Nevertheless, according to White et al. ([1]) it may either increase or decrease, while Thomas ([12]) reported small increments after prolonged standing. As very little ammonia was excreted in these experiments it was thought advisable to make changes more clearcut in our observations by increasing ammonia production through previous administration of ammonium chloride. Furthermore an opportunity was thus afforded to study factors capable of influencing ammonia excretion in fixed acidosis during periods in which no significant changes in acid-balance were registered.

Ammonia excretion decreased in all subjects when they were changed from the recumbent to the semivertical, standing or sitting position. Excretion of titratable acid and phosphates also decreased.

Material and methods

Twelve adults, nine males and three females, aged 20 to 52 with no evidence of previous renal disease were selected and divided for study purposes in four groups of three subjects each. Case 4 had and interatrial septal defect without cardiac failure.

Subjects of group 1 received 10 gm of ammonium chloride (*) on the two days previous to the experiments and 1 gm on the same day upon arising in

(*) Ammonium chloride enteric coated tablets were kindly supplied by Parke Davis, Argentina.

Received for publication, October 15th, 1959.

the morning. They were made to lie on a tilting table as soon as they arrived to the laboratory and the bladder was emptied through an indwelling catheter whereupon zero time was recorded. The postural change in this group consisted in tilting the table to a 60° angle from the horizontal.

Conditions of study were the same for group 2 except that the subjects drank 1000 ml of tap water before starting the experiment.

Groups 3 and 4 differed from groups 1 and 2 in the following:

1) A two hour period of stabilization in the recumbent position was instituted prior to the beginning of the experiment.

2) During the previous four days the subjects received 10 gm of ammonium chloride and 2 gm on the same day, 1 gm upon arising in the morning and 1 gm before lying down. From the assumption of recumbency till the end of the experiment they drank, at twenty minutes intervals, 0.1 ml/Kg of body weight/minute, of a 1.4 % sodium chloride solution.

3) In group 3 standing up was substituted for tilting.

4) In group 4 sitting up was used as postural change.

Experiments of groups 1 and 2 were devised to promote maximal changes in glomerular filtration rate. In fact, it has already been demonstrated that assumption of the supine position is attended by diuresis and increase of creatinine clearance ([2, 13]). On the other hand, tilting is known to produce a greater fall in glomerular filtration rate (judged by inulin clearance) than standing or sitting ([4]).

The purpose of water administration to subjects of group 2 was to provoke water diuresis simultaneously with the tilting procedure. It was thus expected to dissociate the effects of changes of water and salt excretion.

In experiments of groups 3 and 4 it was attempted to reduce to a minimum the changes in glomerular filtration rate by establishing a previous period of stabilization and by substituting standing or sitting for tilting. As presumably, changes in ammonia excretion in these experiments would be of lesser magnitude, it was considered advisable to make them more clearcut by increasing ammonia production. This was attained by prolonging the period of ammonium chloride administration to four days for it is known that ammonia production reaches the maximum about the fifth day ([14]). Moreover, as these experiments were much longer because of previous stabilization, 2 gm of ammonium chloride were given on the same day and a hypotonic saline solution was administered throughout the experiment to avoid excessive water and salt deficits.

Five to six periods were established, ranging in duration from twenty to thirty minutes. Following two or three periods in recumbency, the subjects were changed to the semivertical, standing or sitting position for two additional periods. Except in case 2, the experiments were ended by two periods in the supine position. At the end of each period urine was collected under toluol or vaseline. After each postural change blood samples were drawn under anaerobic conditions from the brachial artery (groups 1 and 2) or an antecubital vein (groups 3 and 4) through and indwelling needle.

Blood pH was measured at 37° and urine pH at room temperature by means of a Cambridge pH meter. Sodium and potassium were determined with a DU Beckman flame photometer, after precipitating plasma proteins by a trichloroacetic-iso-propilic mixture ([15]). Chloride was determined by Scribner's modification ([16]) of Shales's mercury titration method. Ammonia was determined by aereation ([17]), titratable acidity by the Henderson and Palmer method ([18])

and phosphorus by the Fiske method ([19]). CO_2 content of whole blood was measured manometrically. From these data, arterial pH and oxygen capacity, pCO_2, plasma carbonic acid and bicarbonate concentration were calculated ([20]). Creatinine chromogen was determined by the Taussky technique, modified by Brod and Sirota ([21]). Clearance of creatinine chromogen was used as an approximation to glomerular filtration rate.

Results

Results are shown in tables I to V.

During the initial periods in recumbency high values of creatinine clearance were recorded in case 3 of group 1 (table I), in cases 4 and 5 of group 2 (table II) and in case 8 of group 3 (table III). Change to the semivertical, standing or sitting position was attended in all subjects by fall in creatinine clearance and decrease in sodium, chloride, potassium, inorganic phosphates, titratable acid and ammonia excretion (fig. 1 and 2). These effects persisted and were often intensified during the first subsequent period in recumbency, partially or wholly receding during the second one. The previous ingestion of 1000 cc of water partially or wholly prevented postural oliguria without affecting the fall in creatinine clearance or the decline in electrolyte or ammonia excretion.

Fall in creatinine clearance ranged from 27.6 to 92.9 %. Maximal fall (to 15 cc per minute) occurred in case 3 and was attended by dizziness, fainting, nausea and circulatory collapse. Chloride excretion decreased from 29.4 to 91.4 %, sodium from 39.2 to 91.6 %, potassium from 31.9 to 71 %, titratable acid from 27.6 to 83.8 %, ammonia from 17 to 90.1 % and inorganic phosphates from 38.5 to 89.8 % (table 5). Maximal percent fall in creatinine clearance and electrolyte excretion, except phosphates, were found in groups 1 and 2 and minimal in groups 3 and 4. Nevertheless, the number of subjects in each group was too small and the scattering of data too great for statistical evaluation.

Though ammonia excretion fell together with sodium and chloride excretion and with creatinine clearance, there was no close parallelism between the changes of these functions. Thus, ammonia excretion increased while sodium and chloride decreased in the fifth period of cases 1 and 8, in the fourth period of cases 2 and 6 and in the third period of case 10. Conversely, ammonia excretion decreased while sodium and chloride increased in the second period of case 11. Similarly, ammonia excretion increased while creatinine clearance decreased in the second period of cases 9 and 12, in the third period of cases 4 and 10, in the fourth period of case 6 and in the fifth period of case 4. Conversely, ammonia excretion decreased while creatinine clearance increased during the second period of case 6.

Urinary pH ranged from 4.67 to 6.10, small and unpredictable changes occurring during the experiments.

In all subjects, a mild to moderate hyperchloremic acidosis was found which did not change significantly during the experiments.

Discussion

The fall in ammonia excretion in chloride loading experiments was unrelated to changes in blood pH, plasma electrolyte concentration or diuresis and, most probably, depended on the pattern of electrolyte excretion. In these experiments,

TABLE I

Group 1: nonhydrated, tilted subjects

Case	Postural Change	URINE										PLASMA						
		Time min.	pH	Vol. ml/min.	micro Eq/min.					micro gm/min.		pH	mmHg	mM/L	mEq/L			
					Tit. acid.	NH_4	K	Na	Cl	P	C_{CR}		pCO_2	CO_3H_2	CO_3H	Cl	Na	K
	Supine	33	5.07	1.18	37	244	120	212	304	—	153							
1	Supine	60	5.10	1.10	41	219	101	231	305	—	144							
C. R.	Supine	123	5.10	0.90	33	144	85	194	264	—	110	7.39	41	1.29	24	112	142	3.7
	Tilting	165	5.36	0.31	13	53	41	57	85	—	50							
	Tilting	193	5.52	0.375	—	90	71	43	78	—	81	7.38	39	1.22	21.5	116	142	4.0
	Supine	30	4.64	3.90	33	91	137	378	581	366	100							
	Supine	61	4.67	8.65	35	78	137	311	649	275	126	7.41	32	0.97	20	113	143	3.7
2	Tilting	91	4.73	0.66	20	53	47	78	155	167	75							
B. C.	Tilting	111	4.90	0.55	20	69	62	59	150	225	92	7.42	31	0.87	19	111	143	3.6
	Supine	131	4.84	0.53	24	64	67	41	143	254	90							
	Supine	151	4.74	0.85	31	89	83	91	215	331	104	7.41	32	0.97	20	112	142	3.6
	Supine	23	5.18	4.78	37	81	82	621	693	464	211							
	Supine	41	4.98	1.55	19	42	48	372	434	212	103	7.36	39	1.18	21	110	139	3.9
3	Tilting	61	5.06	0.8	10	25	27	390	248	—	47							
R. G.	Tilting	91	—	0.2	6	8	27	52	60	181	15	7.36	34	1.03	18.5	113	141	3.8
	Supine	111	5.72	0.45	10	14	48	74	121	115	77							
	Supine	131	5.72	0.50	20	17	93	85	110	321	122	—	—	—	—	113	138	4.1

Vol.: Urine volume.
Tit. acid.: titratable acidity.
C_{CR}: Creatinine clearance.

TABLE II

Group 2: overhydrated, tilted subjects

Case	Postural Change	URINE									C_{CR}	PLASMA						
		Time min.	pH	Vol. ml/min.	micro Eq/min.					micro gm/min.		pH	mmHg	mM/L	mEq/L			
					Tit. acid.	NH_4	K	Na	Cl	P			pCO_2	CO_3H_2	CO_3H	Cl	Na	K
	Supine	20	5.30	9.80	70	121	180	1400	814	1721	306							
	Supine	42	5.61	10.27	28	31	90	230	360	415	102	7.40	42	1.11	22.1	112	143	3.8
4	Tilting	62	5.80	17.35	36	41	111	310	434	355	99							
G. B.	Tilting	82	5.40	13.05	25	22	64	220	287	176	70	7.41	37	1.09	22.7	112	144	3.9
	Supine	102	5.50	11.60	24	35	62	220	302	238	62							
	Supine	122	4.85	3.75	19	31	54	510	318	181	72	7.41	37	1.09	23.6	112	144	3.9
	Supine	20	5.62	1.40	22	101	101	288	456	434	205							
	Supine	42	5.75	4.72	26	105	132	340	514	430	177	7.38	41	1.22	21.5	112	140	4.0
5	Tilting	64	6.20	14.50	38	80	101	188	370	319	160							
C. B.	Tilting	84	5.61	1.90	10	49	49	110	181	176	69	7.40	39	1.17	21.8	110	140	4.0
	Supine	106	5.33	0.95	12	59	55	116	208	103	116							
	Supine	127	5.50	2.37	20	71	81	161	350	320	152	7.40	41	1.24	21.8	110	140	4.0
	Supine	24	5.30	8.25	32	50	128	396	520	293	124							
	Supine	44	5.90	14.25	39	43	152	399	485	228	136	7.40	41	1.24	23.6	113	141	4.3
6	Tilting	71	6.10	14.00	53	37	156	294	425	157	117							
P. S.	Tilting	91	6.06	13.75	50	41	126	261	330	112	92	7.41	41	1.17	23.0	111	142	4.5
	Supine	115	5.80	4.33	12	28	73	147	203	51	71							
	Supine	135	5.30	4.00	25	38	76	368	482	105	125	7.41	40	1.20	24.0	113	143	4.5

TABLE III

Group 3: moderately hydrated, standing subjects

Case	Postural Change	URINE										PLASMA						
		Time min.	pH	Vol. ml/min.	micro Eq/min.					micro gm/min.	C_{UR}	pH	mmHg pCO_2	mM/L CO_3H_2	mEq/L			
					Tit. acid.	NH_4	K	Na	Cl	P					CO_3H	Cl	Na	K
7 J. R.	Supine	15	5.15	3.13	31	94	137	520	716	590	128							
	Supine	32	5.31	8.11	32	97	138	520	705	573	137	—	—	—	—	108	140	4.4
	Standing	50	5.55	10.0	30	73	170	320	510	540	97							
	Standing	70	5.16	1.47	20	62	105	120	261	453	75	—	—	—	—	107	140	4.2
	Supine	90	5.13	1.0	24	64	100	131	274	—	108							
	Supine	110	5.22	3.2	30	158	121	282	433	610	133	7.37	43	1.28	23.0	108	140	3.8
8 A. A.	Supine	20	5.25	15.2	38	96	117	608	775	340	156							
	Supine	40	5.02	12.05	39	96	120	687	843	321	153	7.39	39	1.23	23.2	109	140	4.4
	Standing	60	4.90	6.0	34	98	108	564	720	312	147							
	Standing	80	4.96	1.82	23	77	83	276	407	212	114	7.39	37	1.30	23.8	109	139	4.3
	Supine	100	4.91	1.45	22	84	82	227	372	246	155							
	Supine	130	4.92	2.40	27	89	108	285	444	380	182	7.40	41	1.19	22.5	109	140	4.4
6 C. C.	Supine	20	—	6.47	48	99	55	362	469	210	114							
	Supine	40	—	8.10	61	128	57	405	510	282	96	7.35	37	1.10	19.3	115	140	3.8
	Standing	60	—	4.05	43	85	46	295	405	202	72							
	Standing	80	—	1.10	11	68	27	180	247	210	49	7.34	35	1.04	18.0	114	140	3.8
	Supine	102	—	1.14	20	71	30	228	272	262	83							
	Supine	122	—	1.45	28	91	36	270	357	377	98	7.34	34	1.03	17.7	113	140	4.0

TABLE IV

Group 4: moderately hydrated, sitting subjects

Case	Postural Change	URINE										PLASMA						
		Time min.	pH	Vol. ml/min.	micro Eq/min.					micro gm/min. P	C_{CR}	pH	mmHg pCO_2	mM/L CO_3H_2	mEq/L			
					Tit. acid.	NH_4	K	Na	Cl						CO_3H	Cl	Na	K
	Supine	20	—	5.45	27	80	47	278	387	112	107							
	Supine	40	—	8.45	25	87	44	296	380	115	104	7.36	45	1.37	24.0	110	140	4.0
10	Sitting	60	—	9.1	29	94	47	268	364	82	92							
E. L.	Sitting	80	—	5.26	23	78	38	242	313	127	81	7.37	44	1.32	23.5	110	139	4.0
	Supine	100	—	3.0	21	79	40	180	273	170	112							
	Supine	121	—	5.34	26	90	71	242	318	192	121	7.37	42	1.27	23.0	110	139	4.0
	Supine	21	6.28	7.19	39	88	82	345	475	300	90							
	Supine	41	6.28	8.75	43	84	79	367	481	453	88	7.37	50	1.52	21.3	109	143	4.4
11	Sitting	62	6.29	7.33	36	68	89	328	440	442	89							
M. R.	Sitting	83	6.35	4.23	31	56	51	207	292	330	73	7.37	48	1.47	20.6	108	143	4.3
	Supine	103	6.18	2.0	29	78	81	256	356	552	100							
	Supine	123	6.25	4.75	38	80	85	312	441	608	101	7.37	44	1.32	19.0	108	143	4.2
	Supine	21	4.83	12.75	95	57	71	242	318	682	94							
	Supine	43	5.04	6.68	60	61	89	220	300	681	89	7.39	48	1.47	25.5	108	144	4.0
12	Sitting	63	5.24	5.75	72	50	45	121	175	546	74							
H. S.	Sitting	84	4.97	1.78	32	44	42	89	146	564	75	7.38	50	1.53	27.0	109	144	4.2
	Supine	104	4.88	1.48	27	38	66	76	117	481	65							
	Supine	126	4.97	6.27	56	69	117	169	229	880	112	7.40	47	1.42	26.7	109	144	4.0

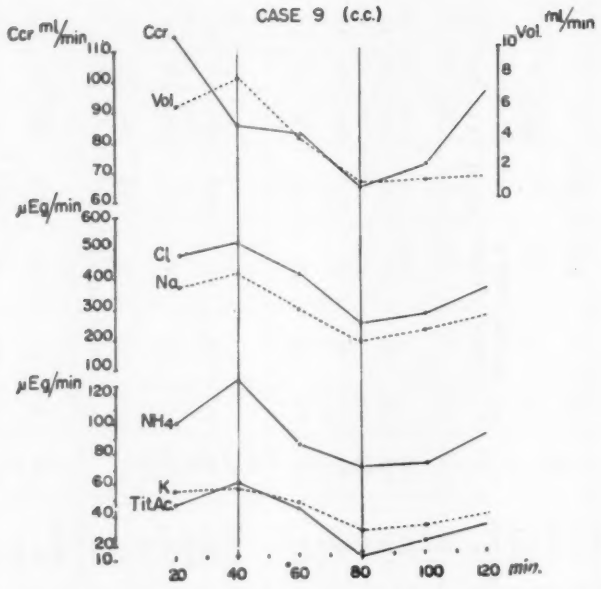


FIG. 1.—*Postural effects in ammonium chloride loading. Fall in creatinine clearance and electrolyte excretion.*

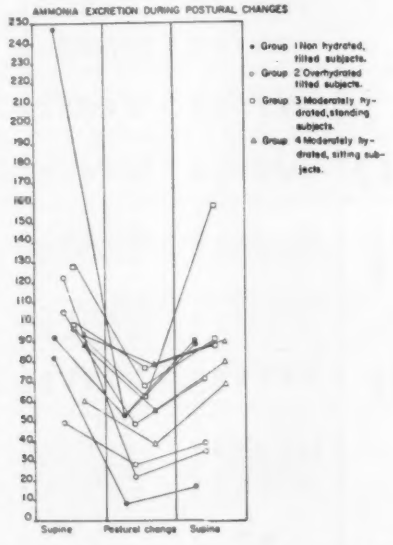


FIG. 2.—*Changes in ammonia excretion during postural changes.*

change from recumbency to the semivertical, standing or sitting position was followed by fall in creatinine clearance, implying decrease in the filtered sodium and chloride load. Moreover, under the stimulus of postural changes sodium reabsorption becomes more complete and, according to Surtshin and White [11], it takes place mostly in the proximal segments. Chloride, being a reabsorbable anion, most probably passively followed sodium in its proximal reabsorption. As a consequence of the decrease in filtered load and relative increase in proximal reabsorption, less sodium was available to the distal tubules for interchange with ammonia and less chloride remained in the distal tubules as ammonia acceptor.

Schwartz et al. [22] and Epstein et al. [23] have independently demonstrated the possibility of influencing ammonia excretion without changing the acid base balance. Schwartz et al. showed that intravenous infusion of sodium sulphate in salt depleted or DCA treated subjects increased ammonia and titrable acidity of urine. Likewise, Epstein et al. reported increased urinary excretion of potassium and ammonia in subjects under intravenous sodium sulphate loading when they were changed from the recumbent to the standing position. The authors ascribed these findings to the presence in the tubular lumen of unreabsorbed sulphate coupled to a strong stimulus for sodium reabsorption. This stimulus was represented by sodium deficit of DCA in Schwartz's experiments and postural changes in Epstein's experiments.

The opposite pattern of ammonia excretion elicited by postural changes in sulphate loading experiments, compared to chloride loading experiments, can be explained on the basis of different availability of strong anions and sodium to the distal tubules. For unknown reasons, in sulphate loading experiments, creatinine clearance did not fall upon standing as it did in chloride loading experiments. Moreover, sulphate being a poorly reabsorbed anion, opposes to sodium proximal tubular reabsorption. As a consequence of unchanged filtered load and decreased proximal reabsorption, a considerable amount of sodium reached the distal segments to be reabsorbed in exchange for ammonia under the stimulus of postural changes, while sulphate remained in the distal tubular lumen as ammonia acceptor.

On the other hand, in the more prolonged studies by Thomas [12] in which ammonia excretion was observed to slightly increase upon standing, the glomerular filtration rate, evaluated from endogenous creatinine clearance, after a small and transitory fall, returned to previous levels. It would then appear that the greater fall in glomerular filtration rate in our shorter studies is the chief factor determining the decrease in ammonia excretion during the erect position, and the increase in proximal tubular reabsorption was apparently unable *per se* to counteract the effect of increased distal reabsorption.

In other words, it seems as if postural changes influence ammonia excretion through the interaction of two opposed mechanisms: 1) Decreased distal sodium and chloride load secondary to fall in glomerular filtration rate and relative increase in proximal tubular reabsorption, inhibiting ammonia excretion; 2) stimulation of distal sodium reabsorption favoring ammonia excretion. In our experiments decrease in distal load predominated over stimulation of distal reabsorption, the ultimate result being a fall in ammonia excretion. Sodium sulphate would "unmask" (Epstein) stimulation of distal sodium reabsorption by preventing decrease in distal load. The same effect would be seen after prolonged standing.

TABLE V

Percent fall in electrolyte excretion and creatinine clearance (*)

Group	Case	Tit. ac.	NH_4	K	Na	Cl	P	C_{CR}
	1	68.3	78.3	65.8	81.4	74.4	—	67.3
1	2	42.9	41.9	65.6	89.1	78	38.5	40.5
	3	83.8	90.1	71.0	91.6	91.4	75.2	92.9
	4	64.2	74.4	65.6	84.3	64.8	89.8	79.7
2	5	73.7	53.4	62.9	61.8	64.8	76.3	66.4
	6	77.3	44	53.2	63.1	61	82.6	47.9
	7	37.5	60.8	41.2	76.9	63.5	—	45.2
3	8	43.6	19.9	31.9	67	55.9	44.2	37.4
	9	58.3	47	52.6	55.5	51.5	51.7	54.3
	10	27.6	17	46.5	39.2	29.4	57.3	33
4	11	32.6	36.4	42.7	43.6	39.3	60.7	27.6
	12	71.6	46	64.1	68.6	63.2	45.3	42

(*) Figures are calculated form maximal and minimal values.

In conclusion, ammonia excretion would depend not only on specific stimuli like acidosis but also on acid acceptors reaching the distal tubular lumen, on availability of sodium to distal tubular cells necessary for exchange reabsorption and on stimuli for sodium reabsorption (sodium depletion, postural changes). Incidentally, this approach to the problem would also, at least partially, explain the increase in ammonia excretion under osmotic ([24]) or mercurial diuresis ([25]) for, in both conditions, proximal sodium and chloride reabsorption admitedly decreases.

Summary

Change from recumbency to the semivertical, standing or sitting position was followed in ammonium chloride loaded subjects by fall in creatinine clearance as well as in sodium, potassium, chloride, inorganic phosphates, titratable acid and ammonia excretion.

It is postulated that postural changes influence ammonia excretion through the interaction of two opposed mechanisms, namely, decrease in distal sodium and chloride load and increase in distal sodium reabsorption. The first mechanism predominates when there is a moderate to marked fall in the glomerular filtration rate and the second one when this does not change or slightly decreases.

References

(1) White, H. L., Rosen, I. T., Fischer, S. S. and Pitts, R. F.: *Amer. J. Physiol.*, 1926, *78*, 185.
(2) Ni, T. G. and Rehberg, P. B.: *J. Physiol.* (Lond.), 1931, *71*, 331.
(3) Brun, C., Knudsen, E. O. E. and Raaschou, F.: *Acta med scand.*, 1945, *122*, 315.
(4) Brun, C., Knudsen, E. O. E. and Raaschou, F.: *J. clin. Invest.*, 1946, *25*, 568.
(5) Kattus, A. A., Sinclair-Smith, B., Genest, J. and Newman, E. V.: *Bull. J. Hopkins Hosp.*, 1949, *84*, 344.
(6) Epstein, F. H., Goodyer, A. V. N., Lawrason, F. D. and Relman, A. S.: *J. clin. Invest.*, 1951, *30*, 63.
(7) Mueller, C. B., Surtshin, A., Carlin, M. R. and White, H. L.: *Amer. J. Physiol.*, 1951, *165*, 411.

(8) Goodyer, A. V. N. and Seldin, D. W.: *J. clin. Invest.*, 1953, *32*, 242.
(9) Holland, B. C. and Stead, E. A.: *J. clin. Invest.*, 1954, *33*, 132.
(10) Pearce, M. L. and Newman, E.: *J. clin. Invest.*, 1954, *33*, 1089.
(11) Surtshin, A. and White, H. L.: *J. clin. Invest.*, 1956, *35*, 267.
(12) Thomas, S.: *J. Physiol.* (Lond.), 1957, *139*, 337.
(13) Rosenbaum, J. D., Nelson, W. P. III, Strauss, M. B., Davis, R. K. and Rossmeisl, E. C.: *J. clin. Invest.*, 1953, *32*, 394.
(14) Wood, F. J. Y.: *Clin. Sci.*, 1955, *14*, 81.
(15) Albert, L. Chaney Laboratories, Glendali, California: *New Flame Photometric Method for Sodium and Potassium in Serum. Application data, DU - 12 - B.*, Beckman.
(16) Scribner, B. H.: *Proc. Mayo Clin.*, 1950, *25*, 209.
(17) Van Slyke, D. D. and Cullen, G. E.: *J. biol. Chem.*, 1916, *24*, 117.
(18) Henderson, L. J. and Palmer, W. W.: *J. biol. Chem.*, 1914, *17*, 305.
(19) Fiske, C. H. and Subbarow, Y.: *J. biol. Chem.*, 1925, *66*, 375.
(20) Van Slyke, D. D. and Neill, J. M.: *J. biol. Chem.*, 1924, *61*, 523.
(21) Brod, J. and Sirota, J. H.: *J. clin. Invest.*, 1948, *27*, 645.
(22) Schwartz, W., Jenson, R. and Relman, A. S.: *J. clin. Invest.*, 1955, *34*, 673.
(23) Epstein, F. H., Kleeman, C. R., Lamdin, E. and Rubini, M. E.: *J. clin. Invest.*, 1956, *35*, 308.
(24) Beck, R. N.: *Clin. Sci.*, 1958, *17*, 37.
(25) Wetson, R. E., Grossman, J. and Leiter, L.: *J. clin. Invest.*, 1951, *30*, 1262.

ON THE INTEGRATION OF RESPIRATORY MOVEMENTS *

J. García Ramos

(Laboratorio de Fisiología, Escuela Médico Militar, Mexico D.F., Mexico)

The current concept on the integration of the respiratory movements assumes a fundamental automatic activity of some neurons in the reticular formation of the medulla oblongata, the so called respiratory center. These cells would be distributed into two groups, one of them driving inspiration and the other controlling, when necessary, the expiratory movements. According to this theory, out of the periodic inhibitory influence of the so called pneumotaxic center and of some of the vagal afferents upon the inspiratory center, it is postulated the existence of direct inhibitory linkages between the inspiratory and the expiratory centers which would provide for the inhibition of one group of respiratory muscles during the contraction of those of the opposite function (Pitts, 1946). This reciprocal inhibition has been established by the facts that inspiration alternates with expiration; that inspiration is maintained, without interruption by expiratory movements, for all the time of stimulation of the inspiratory center and, conversely, that stimulation of the expiratory center leads to a reduction in inspiration (Pitts, Magoun and Ranson, 1939). Since it is generally accepted that during eupneic breathing the only active part of respiration is inspiration, the question may be raised of how a quiescent expiratory center exerts an inhibitory influence.

Another point of view has been expressed. It can be formulated in the words of Marshall Hall (1863): "The rhythm of respiration results largely, if not entirely, from a combination of reflex and supramedullary drives acting on a medullary center functioning more as an integrator of inflowing excitation than as a generator of spontaneous discharge." This idea has found new support with the increasing body of evidence that considers the mid-brain reticular

(*) This investigation was sponsored by the "Fundación para el Fomento de la Investigación Científica en la Escuela Médico Militar A. C.."

Received for publication, August 20th, 1959.

formation as an important center of integration of afferent information. Most recent papers in support of this view are referred to in the excellent review of Heemstra (1956). Another approach to the problem are the recent findings of afferent nerve fibres which may have a selective influence on either inspiration or expiration (Widdicombe, 1954; Paintal, 1955).

The vast majority of the papers on the subject analyzes the problem mainly from the side of inspiratory movements. The present paper deals with some aspects of the reflex control of respiration. Here, expiratory activity has been studied in some detail, as it was felt that this was necessary in order to have a more close understanding of the problem.

Methods

The observations were made mostly on rabbits, although cats and dogs were also used without finding important differences. All animals were under pentobarbital anesthesia which was never too deep. A three-way tracheal cannula was used. On one of its branches a thermocouple (constantan copper) was inserted to have an index of ventilation in the electrical records. Another branch was used for the application of either positive or negative pressure into the lung, as described in a previous paper (García Ramos, 1956). Applied pressures were read at a side tubing on the system, at some distance from the cannula. As the third branch of the cannula was left open to permit the free movement of air into and out of the lung, the pressures read at the manometer do not represent the actual intrapulmonary pressure. (On a model made with a surgeon glove the pressure inside the glove was less than half that of the mercury manometer reading.)

The muscle fibers of the diaphragm which are inserted on the xiphoideal appendix were exposed and small steel forceps suspended by flexible wires were used as electrodes for myographic recording. Similar electrodes were used for expiratory muscles, sometimes the intercostals, but usually the abdominal muscles at the higer portion of the lateral walls. In many cases, the electromyographic activity was electrically integrated on a Grass Electromyograph and Integrator, and recorded on a Grass Polygraph. The muscle potentials and the thermocouple records were taken either on a Schwartzer E. E. G. or on the Grass Polygraph. The stimulator employed was a square pulse generator (Grass S 4). In open chest animals, lung ventilation was maintained by a variable stroke, intermittent positive pressure pump.

Results

A) *Some characteristics of expiratory activity.* — In most cases it was possible to record electromyographic activity from the expiratory muscles, provided the anesthesia was not too deep. Sometimes expiratory discharges appear as small groups, in general toward the middle of the expiratory period. In some other cases there is a continuous tonic activity whose frequency increases periodically either during expiratory movements or during inspiration. Abdominal muscles ordinarily give activity of the first type (see the figures). Internal intercostal muscles often show the second pattern of activity, not rarely presenting the increase in frequency of discharges during the inspiratory phase. Intermediate steps between these two are also seen.

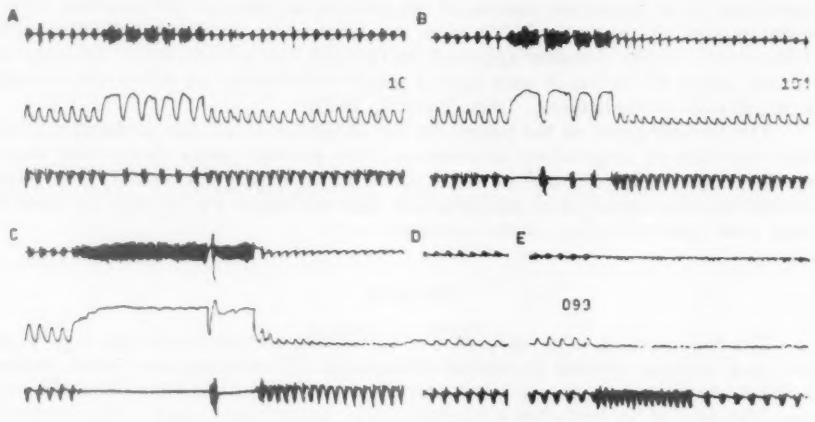


FIG. 1. — *Effects of inflation and deflation of the lung on expiratory and inspiratory activities. Rabbit, closed chest, dead space slightly increased.*

A. — Inflation of the lung during 10 seconds (3 mm Hg positive pressure). Upper tracing, electromyogram of the abdominal muscles. Middle tracing, the same electrical activity integrated. Lower tracing, electromyogram of the diaphragm.

B. — As in A. Greater inflation (5 mm Hg).

C. — With still greater positive pressure (10 mm Hg). After inflation expiratory activity has gradually decreased almost to zero, and persists in reduced form for about 5 minutes.

D. — Eight minutes after C. Inspiration has returned to control values. Expiration is still reduced.

E. — Ten minutes after C. Expiratory activity was abolished by deflation of the lung (— 5 mm Hg). The effect persisted for more than 5 minutes.

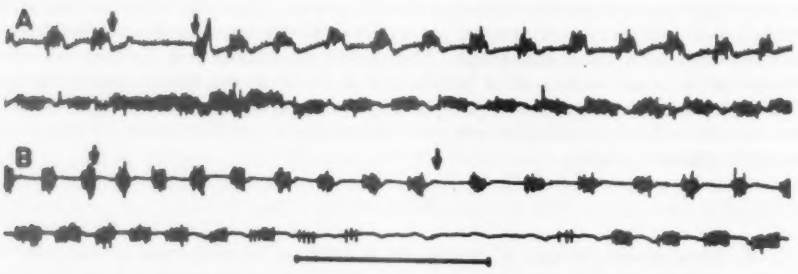


FIG. 2. — *Effects of inflation and deflation of the lung. Rabbit, closed chest. Spontaneous expiration during eupnoea. Electromyogram of the diaphragm, upper record, of the abdominal muscles, below.*

A. — Expiratory muscle potentials increase and may occur during inspiration following a short period of strong inflation of the lung. Between the arrows a positive pressure of 9 mm Hg was applied.

B. — Gradual reduction of expiratory activity during deflation of the lung (— 2 mm Hg between the arrows). Recovery is also gradual.

The horizontal line at the bottom represents 10 seconds.

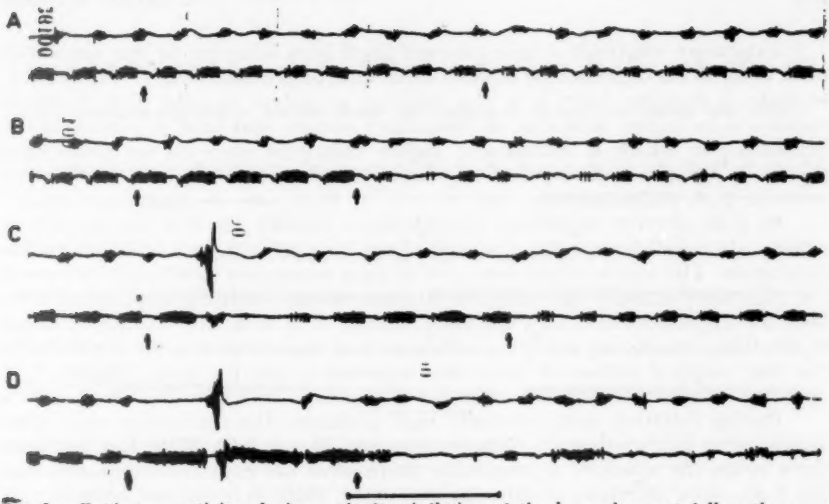


FIG. 3. — *Expiratory activity, during and after inflation of the lung, does not follow the same temporal course than the inspiratory one. Same records as in figure 2. Rabbit, eupnoeic breathing.*

A. — Between the arrows a sligth positive pressure was applied (0.5 mm Hg). The expiratory response shows some accommodation and a rebound after inflation.

B. — The rebound appears clearly after a greater inflation (3 mm Hg).

C. — Depression of the response to inflation after a strong inspiratory movement of the animal.

D. — A stronger inflation of the lung (between the arrows a positive pressure of 5 mm Hg was applied) may give an after reduction of expiratory activity. During this period expiratory movements may become uncoördinated with inspirations.

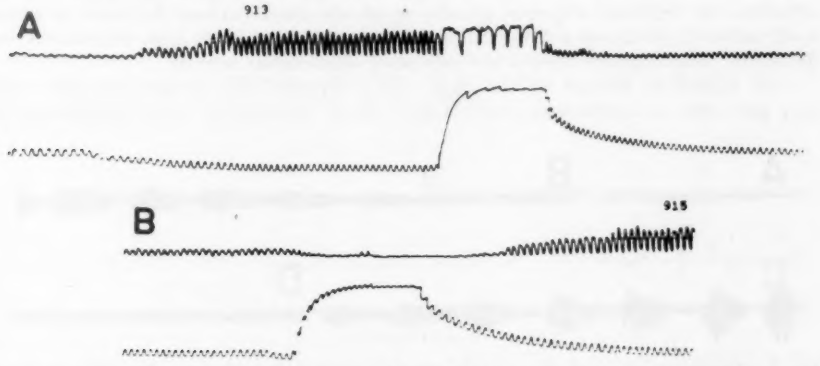


FIG. 4. — *Some characteristics of expiratory activity. Rabbit. Integrated electrical activity of the abdominal muscles (upper tracings). The lower records are from a thermocouple inserted at the tracheal cannula.*

A. — At the point in which the thermocouple tracing shows a downward deflection, the dead space of the airways increased. At the upward deflection, the lung was inflated with a pressure of 5 mm Hg.

B. — Is a continuation of A. *The upward deflection in the lower tracing marks the deflation of the lung with a negative pressure (— 5 mm Hg). The depressed expiratory activity following inflation was recovered after deflation.*

Expiratory electrical activity showed itself very sensitive to the anesthetic used. Once it was depressed by an additional dose of pentobarbital, it was difficult to make it reappear later. It is also sensitive to carbon dioxide. The threshold appears to be higher than that of inspiratory activity, but once it is reached the proportion in which it increases, is higher than that observed for inspiration (figure 4). Oxygen lack depresses expiratory activity which appears to be more sensitive than the inspiratory one.

B) *The afferent regulation of expiratory activity.* a) *The Hering-Breuer reflex.* — It is well known that inflation of the lung inhibits, and deflation excites respiration. The way in which every one of these maneuvers specifically influences the expiratory activity has been less studied. When recording respiration from both an inspiratory and an expiratory muscle, it is seen that during inflation of the lung, inspiratory activity is inhibited and expiratory activity is enhanced, but the temporal course of those two responses is not the same (figures 1, 2 and 3). There are also differences in the responses to deflation (figures 1 and 2).

During inflation with relatively high pressures, the continuous expiratory activity that follows shows a clear recruitment (figure 1 *C*). With low pressures there is, on the contrary, a progressive decrease of the expiratory response (figure 3 *A*). After inflation, if this was prolonged, there is a marked reduction of expiratory activity reaching a maximum some time after the period of inflation (figures 1 *A*, *B* and *C*; and 3 *A*, *B* and *C*). The reduced activity may last for several minutes (figures 1 *C* and *D*; and 4). If the inflation is of short duration, however, there is an increase of activity over the basal values. In this last case expiratory activity may occur also during some of the inspiratory phases which follow the inflation period (figure 2 *A*).

With deflation of the lung, the proportion by which expiratory activity is depressed is much higher than that by which inspiration is enhanced (figures 1 *E* and 2 *B*). In all cases the amplitude of the responses of expiratory activity to inflation or deflation depends greatly upon the basal values. If there is great basal activity, it shows a large increase with inflation and light inhibition by deflation. The opposite results are seen with small basal activity.

b) *Effects of section of the vagi.* — It is known that section of both vagi may give rise to continuous inspiratory activity recorded as tonic discharges of

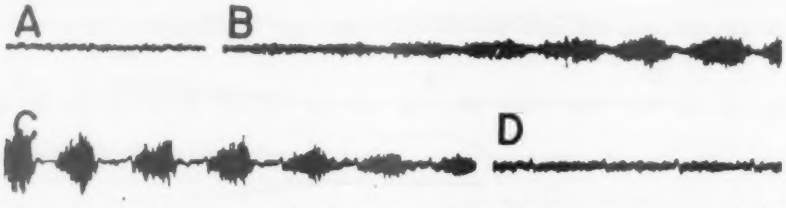


FIG. 5. — *Inspiratory apneuses after double vagotomy changed into rhythmic breathing by hypoventilation. Rabbit under artificial respiration. Electromyogram of the diaphragm.*
A. — *Tonic basal activity of the diaphragm. Lung ventilated with 45 ml of air, 24 times per minute.*
B. — *Twenty seconds after reduction of the volume of ventilation to 25 ml. Breathing movements had the same frequency as the respiration pump.*
C. — *Fifteen seconds after* B. *At the start of the record the volume of air was increased to 75 ml.*
D. — *Twenty seconds after the end of* C.

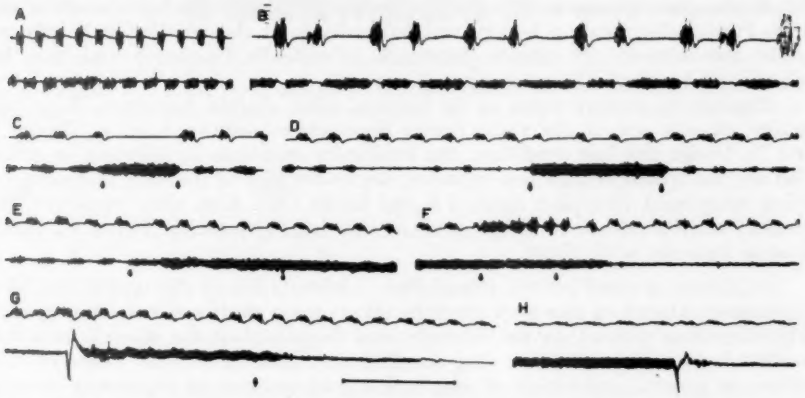


FIG. 6. — *Effects of the section of the vagus and those of stimulation of the central end of the nerve. Respiratory movements made slightly stronger by increasing the dead space.*
A. — Control before cutting the right vagus at the neck.
B. — Ten seconds after the nerve section.
C. — With the amplification reduced for the record of the electromyogram of the diaphragm (upper tracing). Two minutes after vagus section. Between the arrows, the central end of the nerve was stimulated with rectangular pulses 2 msec duration, at 50 per sec, and 0.52 volts.
D. — Six minutes after the nerve section. Expiratory activity has decreased. Between the arrows, the nerve was stimulated as in C *but with a lower intensity (0.15 volts). Note the smaller effect on inspiration.*
E. — Seven minutes afterwards. Between the arrows, stimulation as in D *but with a still lower intensity (0.12 volts). A continuous expiratory activity appeared after stimulation.*
F. — During the following minute after E. *Lung's deflation (— 5 mm Hg) reduced and later abolished the continuous expiratory activity.*
G. — The continuous expiratory activity reappeared after a strong inspiratory movement of the whole animal (See the artifact). At the arrow the increased dead space was suppressed.
H. — Another episode of continuous expiratory activity induced by vagal stimulation as in E_2 *and its disappearance after a movement of the animal.*
Time callibration: 10 seconds

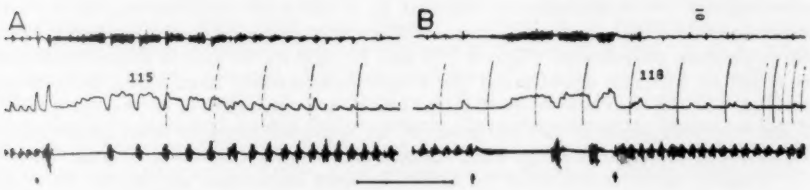


FIG. 7. — *Expiratory activity depends on vagal afferent impulses. Section of one of these nerves reduces the amplitude of expiratory movements and decreases the responses to inflation. Rabbit, same animal as in figure 1.*
A. — At the arrow the right vagus was cut. The changes were the result of the mechanical stimulation during section.
B. — Three minutes later. Between the arrows, a positive pressure of 10 mm Hg was applied. Compare the results with those in figure 1. The inspiratory response is definitely smaller. Time: 10 seconds.

the diaphragm (apneuses). This activity depends closely on the ventilation of the lung. It may disappear in hyperventilated animals. If the ventilation is reduced below the normal, the muscle potentials increase in frequency and may be substituted by rhythmic movements (figure 5).

Expiratory activity tends to be reduced after double vagotomy. Even the section of only one of the vagus nerves depresses expiratory activity (figures 6 and 7). Under this last condition, the expiratory responses to inflation or deflation are less apparent than was expected, since only half of the lung receptors are being stimulated (compare figure 7 *B* and figure 1 *C*). Also, after vagotomy, expiratory activity may loose the property of showing rhythmicity (figure 6). There is some recovery with time.

c) *Effects of vagal central stimulation.* — Stimulation of the central end of a cut vagus is known to give very complex effects upon the respiratory movements. The variations depend on the intensity and frequency of the stimuli, and this is what is to be expected knowing the different kind of afferent fibers in that nerve. In general, inhibition of inspiration and increase of expiratory activity are the predominant effects. With a certain frequency and submaximal intensity of stimulation it was possible to obtain rhythmic breathing from apneuses induced by double vagotomy. The frequency of this rhythmic breathing may depend on the rhythm of the chest wall movements produced by the artificial respiration. For example, stimulating with a frequency of 50 per second may give rhythmic breathing exactly following the chest wall movements, inspiratory activity appearing after each pump stroke. By increasing the frequency of stimulation of the vagus from 50 to 60 per second the rhythmic activity of the diaphragm fell to half the previous one.

Vagal stimulation effects on expiratory activity were explored shortly after vagotomy and with the lung hypoventilated or with increased dead space. Stimuli which induce inhibition of inspiratory movements, simultaneously induce an increase in expiratory activity. This increase has a lower threshold than the inhibition of the inspiration. An intensity of stimulation can be selected that gives almost pure expiratory response (figure 6 *D* and *E*).

d) *Effects of stimulation of somatic afferent nerves.* — From all the somatic afferent nerve trunks whose stimulation was tested it was possible to obtain some influence on respiratory activity. The responses were complex and in most cases afferent nerve stimulation affected in a different way the expiratory and inspiratory activities. In some instances the effects were almost exclusively upon the expiratory movements (figures 6 *G* and *H*; and 8). In fact it is more difficult to affect by afferent stimulation the diaphragm activity than other inspiratory muscles. Feeling that one of the factors which may contribute to the complexity of the responses could be the influence of the vagal afferents, in some observations the afferent fibers stimulated were tested after double vagotomy (figure 9).

Somatic nerve stimulation no doubt induces important alterations on respiratory movements. To have an idea of the possible role this afferent impulses may have on the control of normal breathing, a more physiological way of stimulation was tested by displacing the thoracic or the abdominal walls. It was found that expansion of the thoracic wall enhances, and collapse of those walls, as well as with compression of the abdominal ones, depresses inspiratory movements. Those effects are opposite in sign to the responses obtained with those maneuvers in animals with intact vagi (figure 10). On expiratory activity the opposite effects are seen, the muscle potentials appearing also during the inspi-

FIG. 8. — *Effects of a single shock applied to the saphenous nerve. Cat. Electromyogram of the abdominal muscles (upper record) Thermocouple in the tracheal cannula (lower record). Note the greater effect on expiration.*

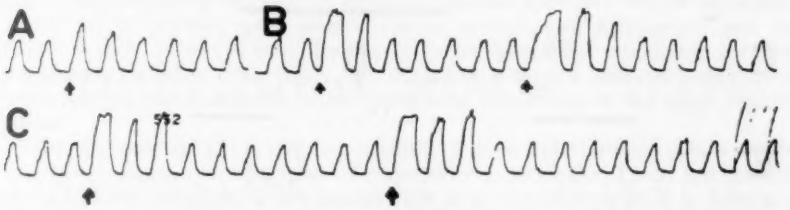


FIG. 9. — *Influence of somatic afferent stimulation. Rabbit, double vagotomy. Integrated electrical activity of the diaphragm.*
A. — At the arrow, tactile stimulation at the right posterior leg.
B. — At the first arrow, a light tap was applied to the knee, as in exploring the patellar reflex. At the second arrow, four of those taps were applied during two seconds.
C. — At the first arrow, tactile stimulation of the anterior part of the belly. At the second arrow, the same type of stimulation was applied to the chest wall.

ratory phases (figure 11). All the responses to afferent stimulation depend on the basal level of activity and also closely on the ventilation of the lung. They are of smaller magnitude if the animal is hyperventilated.

DISCUSSION

Let us make first a brief summary of the observed facts. *a*) Lung inflation gives rise to an increase in expiratory activity (figures 1, 2, 3, and 7). *b*) Expiratory response to inflation shows recruitment with stronger inflations and accommodation with smaller inflation, expiratory activity may be depressed. This depression is greater the higher the pressure of inflation or the longer the period of it. Its maximum occurs some time later (figures 1, 3 and 4). *d*) After vagal central stimulation expiratory activity may be enhanced (figures 2, 6 and 7). *e*) Section of the vagus nerves strongly depresses expiratory activity (figures 6 and 7). *f*) Inspiratory activity responses to inflation, deflation, or direct vagal stimulation are opposite in sign but do not follow closely the same temporal course or have the same proportional changes as those of expiratory activity. *g*) Expiratory activity is more sensitive to inhibitory and to nervous facilitatory influences. *h*) Somatic afferent stimulation has important effects on respiratory movements particularly on expiration. *i*) Movements of the chest and abdominal walls have a control on respiration (figures 5, 8, 9 and 10). These effects are opposite in sign to those depending from the Hering-Breuer reflex.

The present observations give strong evidence of the important role of afferent impulses on the integration of respiratory movements. They also show the complexity of the functional relations between inspiratory and expiratory central drives. The idea of reciprocal inhibitory influences of inspiratory and expiratory

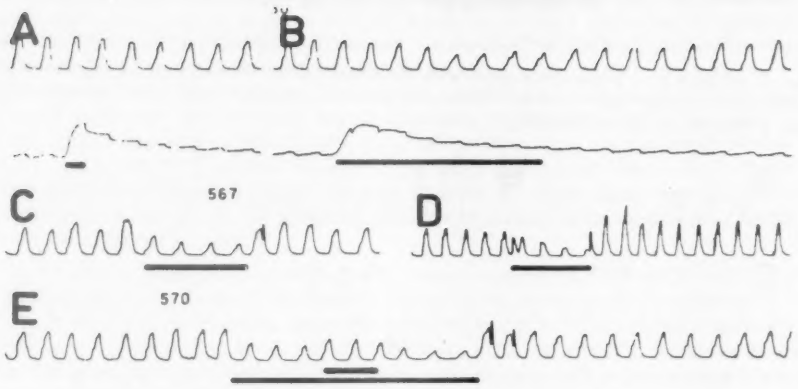


FIG. 10. — *Effects of the chest wall movements on respiration. Rabbit, double vagotomy. Integrated electrical activity of the diaphragm.*

A and B. — Deflation of the lung (— 10 mm Hg) during the period marked by the horizontal heavy line at the bottom. The lower record is from a thermocouple in the tracheal cannula.
C. — Manual compression of the abdomen during the time marked.
E. — Inflation of the lung (small horizontal line) during a period of manual compression of the abdomen (lower horizontal line).
D. — With the chest wide open and under artificial respiration. Lung inflation or collapse did not produce any effect. The record shows the changes during and after manual compression of the chest walls.

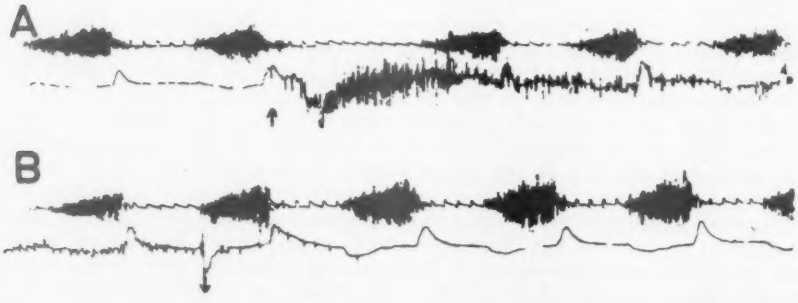


FIG. 11. — *Effects of compression of the chest walls on expiratory activity. Rabbit, double vagotomy. Electromyograms of the diaphragm (above) and of the abdominal muscles (below).*

A. — At the arrow, pressure upon the chest walls was applied.
B. — Is a continuation of A. The arrow marks the moment in which pressure upon the chest was released.

centers appears too simple to explain all the facts. The observation of the possible coexistence of inspiratory and expiratory activities (figures 2, 6 and 11) is, in fact, contrary to that hypothesis. The differential effects of afferent stimulation, and the peculiarities of the responses on the inspiratory and expiratory activities show that every one of them is controlled by a specific set of afferent impulses.

The greater sensitivity of expiratory activity to afferent inhibitory and excitatory influences would be possibly explained if that influence would be exerted at spinal levels. Indeed, such phenomena as recruitment, facilitation and the myotatic stretch reflex (García Ramos & López Mendoza) which can be seen in respiratory muscles other than the diaphragm suggest some integration at spinal level, but this hardly could be applied for the effects of the vagal afferent impulses.

With all these data it is still not possible to draw definite conclusions on the delicate mechanisms of integration of respiratory movements. These mechanisms appear to be too complex. A few points may, however, be presented at least as a working hypothesis. *1.* – Carbon dioxide would be the main driving force for inspiratory as well as for expiratory central discharges. The partial pressure of this gas in the blood would determine the level of central excitability. *2.* – At subnormal levels of central excitability, rhythmic breathing would not be possible due to the lack of effect of the afferent impulses normally reaching the centers (figure 5). With normal excitability of the centers, or with levels above normal, rhythmic breathing would be dependent on afferent control (figures 5, 9 and 10). Vagal afferents are of utmost importance in this respect. They exert a normal tonic influence on expiratory activity (figures 6 and 7). *3.* – Central activity could be modulated by afferent influences upon the spinal motoneurons and for the level of excitation at this point.

Summary

By using electromyographic records of the diaphragm and the abdominal muscles as an index of inspiratory and expiratory central activities, respectively, observations were made on rabbits, cats and dogs under light pentobarbital anesthesia.

1) Both the inspiratory and the expiratory centers are sensitive to carbon dioxide. The expiratory center has a higher threshold.

2) Expiratory movements are readily depressed by anesthesia.

3) Inspiratory and expiratory activities are influenced markedly by afferent impulses. The most important action is that of the vagal lung afferents. Movements of the thoracic and abdominal walls are important for the integration of rhythmic breathing. In animals with double vagotomy, contrary to what happens in the normal animal, enlargement of the thorax excites inspiration and collapse of the thorax or compression of the abdomen inhibits inspiration and excites expiration.

4) Inspiratory and expiratory responses to afferent stimulation do not follow the same temporal course; they do not change in the same proportions, and do not necessarily show reciprocal effects.

It is concluded that carbon dioxide determines the level of excitability of the centers. That afferent impulses are very important in determining rhythmic breathing, and that organization of the respiratory center does not merely depend on reciprocal inhibition of inspiratory and expiratory centers.

Resumen

Se hicieron observaciones en conejos, gatos y perros, bajo anestesia ligera con pentobarbital, empleando los registros electromiográficos del diafragma y de los músculos abdominales como índices de la actividad de los centros inspiratorio y expiratorio, respectivamente.

Tanto uno como otro centros son sensibles a las variaciones del bióxido de carbono de la sangre. El centro expiratorio parece tener un umbral más elevado, es más fácilmente deprimido por el anestésico y su actividad varía marcadamente bajo la influencia de la estimulación aferente.

Los movimientos respiratorios son afectados principalmente por los aferentes vagales. Los movimientos de las paredes torácicas y abdominales son factores importantes en la integración de la respiración rítmica. En animales con sección bilateral de los vagos, contrariamente a lo que sucede en el animal normal, el ensanchamiento del tórax excita la inspiración y la reducción de esta cavidad o la compresión de las paredes abdominales inhibe la inspiración y provoca la expiración.

Las respuestas inspiratoria y expiratoria a la estimulación aferente no siguen precisamente el mismo curso temporal, no muestran variaciones en las mismas proporciones, y no necesariamente muestran cambios de signo contrario.

Se concluye que los impulsos aferentes desempeñan un papel muy importante en la determinación de la ritmicidad de la respiración. La tensión de bióxido de carbono determinaría el nivel de excitabilidad de los centros. La organización del centro respiratorio es mucho más compleja de lo que establece la hipótesis de una simple inhibición recíproca entre los centros inspiratorio y expiratorio.

References

(1) García Ramos, J.: *Amer. Rev. Tuberc.*, 1956, *73*, 519.
(2) García Ramos, J., López Mendoza, E.: *Acta physiol. lat-amer.*, 1959, *9*, 257.
(3) Hall, M.: *Amer. J. Physiol.*, 1954, *178*, 521.
(4) Heemstra, H.: *Ann. Rev. Physiol.*, 1956, *18*, 121.
(5) Paintal, A. S.: *Quart. J. exp. Physiol.*, 1955, *40*, 89.
(6) Pitts, R. F.: *Physiol. Rev.*, 1946, *26*, 609.
(7) Pitts, R. F., Magoun, H. W., Ranson, S. W.: *Amer. J. Physiol.*, 1939, *126*, 687.
(8) Widdicombe, J. G.: *J. Physiol.* (Lond.), 1954, *125*, 336.

ON THE INTEGRATION OF RESPIRATORY MOVEMENTS II. THE INTEGRATION AT SPINAL LEVEL *

J. García Ramos and E. López Mendoza

(Laboratorio de Fisiología, Escuela Médico Militar, Mexico D.F., Mexico)

In the first paper of this series (3) it was described that expiratory muscles play a role in normal breathing, and that somatic afferent stimulation have an important effect on respiratory muscles. As it was shown that after double vagotomy the passive displacement of the thoracic and the abdominal walls could enhance or depress the activity of respiratory muscles, the question was raised of how much of this influence during active normal respiration could play a role in determining or modifying rhythmic breathing in the normal animal, and also, what portion of the reflex activation or inhibition could take place at spinal level.

Both questions, at first sight, look quite heterodox. Indeed, they do not find strong support in the literature. Most authors consider as a universal truth the automatic rhythmicity of the respiratory center. Even if in recent papers the reflex nature of rhythmic breathing is emphasized (1) still the idea dominates that somatic afferent impulses exert their influence directly upon the respiratory center (4, 5, 6). Wang and Wang in a recent review (8) state that "sensory inflow from whatever source tends to reinforce the respiratory center's response to carbon dioxide". On the other hand, Calma (2) has reported the poor effects that a great variety of afferent impulses can have upon the phrenic motoneurons.

Considering, however, that expiratory activity has been less well studied from this point of view, that it has importance on respiratory activity, and that the motoneurons responsible for this kind of movements form also part of the mechanism of somatic movements, it was decided to explore how somatic reflexes may interfere with respiratory movements particularly at the spinal level. This is the subject of the present study.

(*) This investigation was sponsored by the "Fundación para el Fomento de la Investigación científica en la Escuela Médico Militar A. C."

Received for publication, August 20th, 1959.

Methods

The observations were made on rabbits and cats under light pentobarbital anesthesia. Most of the experiments were done on animals in which an incomplete section of the spinal cord was made at C 2. The ventral funiculi were left intact with the idea of maintaining the supposed facilitatory influence of central impulses upon the motoneurons, and of suppressing or reducing to a minimum the arrival to the respiratory centers of the afferent impulses derived from the stimulation and from the respiratory movements themselves. Both vagi were also cut at the neck in most cases. At the end of the experiment the segment of the spinal cord containing the section was soaked for one hour in a 1 per cent solution of Congo Red and then immersed, for 24 hours, in 10 per cent formalin. The colloidal dye diffuses very little into the tissue and permits to see under the dissecting microscope the extent of the section made, mainly because the gray substance stains more deeply than the white matter.

Some other experiments were made on animals with a complete transection of the spinal cord, at the same or other levels. Also, in the above cited preparations, the section was completed later in the course of the experiment, slightly above or below that point. In most cases, the observations were made before one or the other type of spinal section and repeated thereafter.

Respiratory activity was recorded by electromyography of the corresponding muscles: diaphragm, at the muscle fibers which insert at the xiphoideal appendix; external or internal intercostal muscles, usually those at the 8th, 9th or 10th intercostal spaces, along the posterior or midaxillary line; and the oblique abdominal muscles along the same line at the higher portion of the abdomen. As recording electrodes, small hooks of number 34 nichrome wire were used. The hooks were soldered to a very flexible Nº 36 magneto wire to hang them from a support in order to reduce movement artifacts.

The records were taken on ink writers oscillographs, either on a Schwarzer E. E. G. or a Grass Polygraph. To stimulate the nerve trunks tested, a Grass square pulse generator (model S 4) was used. When artificial respiration was required, a Starling type pump was employed. In some cases ventilation was recorded by a thermocouple inserted at the tracheal cannula.

Results

A) *The stretch reflex of the intercostal muscles.* — Stretching of intercostal muscle fibers gives a reflex contraction of them. This reflex contraction occurs in the intact animal and also after section of the spinal cord above the corresponding level of the intercostal space explored (figure 1). The response is proportional to the amount of stretching. Continuous stretching gives a continuous response showing accommodation. In many cases the reflex response could be seen modullated by the respiratory movements of the chest wall (figure 2). Stretching of the muscle fibers was accomplished by fixing the upper rib to a supporting rod and pulling the lower rib with a hook tied to a thread. Two pulleys permitted to adjust the tension exerted on the thread by hanging weights of different magnitude.

With the method employed it was not possible to establish quantitative relations between the tension applied and the amplitude of the contractions.

FIG. 1. — *Stretch reflex of an intercostal muscle showing accommodation and the effects of spinal shock. Rabbit under artificial respiration, with an incomplete section of the spinal cord at C2, leaving the anterior funiculi intact. — A. – At the arrow, pulling apart the corresponding ribs with a tension of 200 g. — B. – After total section of the spinal cord. Between arrows, the tension applied was of 500 g.*

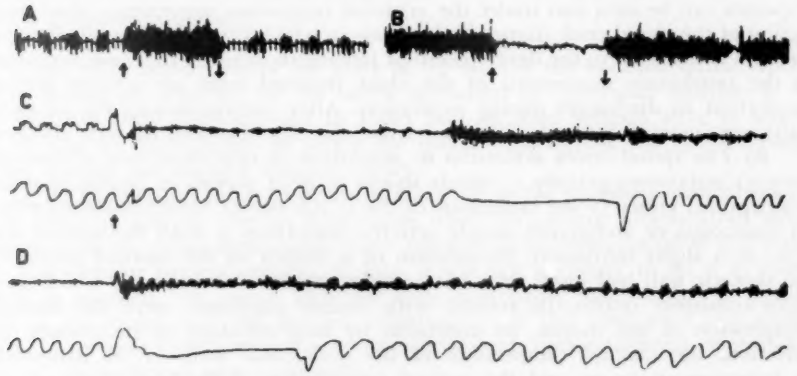


FIG. 2. — *Reflex responses to inflation and deflation of the lung, before and after transection of the cord at T3. Rabbit, intact vagi. Electromyograms of internal intercostal muscles. — A. – Inflation and B deflation of the lung before the section of the spinal cord. — C. – In the spinal animal, at the arrow, the ribs were pulled apart with a tension of 200 g. The lung was inflated later, as before, during the period marked by the stopping of breathing in the lower record from a thermocouple in the tracheal cannula. — D. – Is a continuation of C. The thermocouple record shows the time during which the lung was deflated. In the spinal animal, both inflation and deflation induces reflex activity of intercostal muscles. This activity gives residual facilitation for the stretch reflex which shows rhythmic discharges during the spontaneous diaphragmatic breathing.*

For a given degree of stretching, the response depends upon the level of excitability of the spinal motoneurons involved. This level is greater after facilitation of any kind (figure 2). If the respiratory movements, which can be considered as representing by themselves an afferent rhythmic stimulation, are of greater amplitude, for example by increasing the dead space, its facilitation producing effect increases and this is shown by stretch reflex responses of greater magnitude. The level of excitability is lower in the spinal animal shortly after the spinal section (figure 1) and it also depends on the degree of inhibition that can be induced by some types of complex stimuli (see below).

Other reasons that make it difficult to obtain a quantitative stimulus/response relation are as follows. Injury may be caused to the muscle fibers either

by the electrodes, by the stretching itself or by dessication. It was common to see the responses decline slowly with time. The tension applied is distributed along the whole intercostal space and in a great proportion is going to be taken by the rib joints and by the surrounding tissues. In addition, it is not easy to measure exactly neither the stimulus nor the response. It can be said, however, that the response of these muscles to stretching is of relatively low threshold. The records in figure 1 were taken with a tension on the thread of 200 and 500 grams. In the last case, the actual displacement between the ribs, at the place of the electrodes, was not more than 1 millimeter.

In animals in which diaphragmatic breathing was left by a spinal cord section just below the site of the phrenic motoneurons, it was usual to see rhythmic responses of the intercostal or abdominal muscles following the chest wall movements induced by the active diaphragm (figure 2). In some cases those rhythmic responses can be seen also under the artificial respiratory movements after transection of the spinal cord (figure 3). In a case in which the tension applied to the hook on the ribs led to the development of pneumothorax, the rhythmic responses to the respiratory movements of the chest inverted from an activity during inspiration to discharges during expiration. After pneumothorax the thoracic walls were moving down during inspiration, the ribs approaching each another.

B) *The spinal reflex activation or inhibition of intercostal and abdominal muscle's respiratory activity.* — Single shocks or brief periods of tetanic stimulation, applied either to the saphenous or the sciatic nerves, have almost no effect on intercostal or abdominal muscle activity. Sometimes a small facilitation was seen, or a slight inhibition. Stimulation of a branch of the cervical plexus to the thoracic wall had more clear but complex effects. Other stimuli which gave more consistent results, in animals with double vagotomy, were the manual compression of the thorax, its distension by lung inflation or its collapse by deflation, the manual compression of the abdominal walls or its distension by pneumoperitoneum, and the manual compression of the hind limbs or the tail. It is not easy to make a quantitative evaluation of the magnitude of this kind of stimulation, but it can be said that it was always below the level of producing pain. In fact, the threshold of the responses is very low. Those ma-

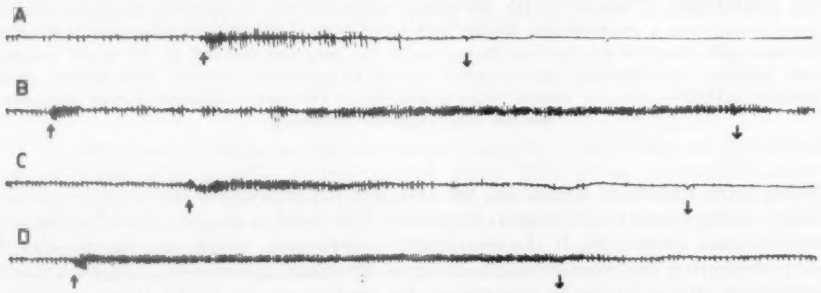


FIG. 3. — *Spinal inhibition is more sensitive than excitation to asphyxia. Rabbit, transection of the spinal cord at C2 and double vagotomy. Animal under artificial respiration. Electromyograms of external intercostal muscle. — A. – Between signals, manual compression of the thoracic walls. The residual inhibition persisted for two minutes. — B. – Manual compression of the belly, between arrows. — C and D. – The same maneuvers as in A and B, respectively, after reduction of the stroke volume to half the previous value. The residual inhibition in C persisted for only 15 seconds. No inhibition is shown in D during the period of stimulation.*

neuvers were selected, particularly the ones including changes in position of the thoracic and abdominal walls, or changes in pressure at the thoracic or abdominal cavities, because they might give an idea of the role that the respiratory movements could have on the activity of the muscles involved during normal respiration.

Most of these maneuvers have a complex effect, activation and inhibition, both in the same response although in different and variable proportions. Usually inhibition follows the increase of activity and probably is a factor in the establishment of accommodation (figures 3, 4, 5, 6 and 7). As after-effects, facilitation or inhibition may occur (figures 2, 6 and 7). The amount of inhibition present in any given response seemed to depend upon ventilation. Inhibition was present in well ventilated animals and reduced or even suppressed reversibly by hypoventilation. Asphyxia reduces inhibition earlier than the positive excitatory responses (figure 3).

Transection of the spinal cord is known to produce important depression of spinal reflex activity below the place of the section, the so-called spinal shock. This depression occurred for the reflex activation of intercostal muscles. It is of relatively short duration and in about half an hour reflexes were as active as seen in the preparation in which the spinal cord section was made incomplete, leaving the anterior funiculi intact.

C) *The effects of somatic afferent stimulation on the phrenic motoneurons.* — The effects of somatic afferent stimulation upon the phrenic motoneurons were explored in the preparation cited above (section of the spinal cord at C 2 leaving the anterior funiculi intact). In fact, it was not necessary to do it as complete as planned, the results obtained were satisfactory when only the dorsal half of the spinal cord was divided. Observations of this kind were also made on animals with total transection of the spinal cord, under artificial respiration (in all cases after double vagotomy).

The conclusion of Calma ([2]) was confirmed, but only in the sense that stimulation of a great variety of nerve trunks have very poor effects upon the phrenic motoneurons. By the use of the complex types of stimulation mentioned above, however, it was usual to demonstrate an important influence of somatic afferents upon those motoneurons (figure 5). The most frequent finding when the impulses of the respiratory center were left to reach the phrenic motoneurons, was that the responses to afferent somatic stimulation were less clear than after suppressing the arrival of these central discharges, either after a complete section of the spinal cord or simply by hyperventilation (figure 6).

In animals with the cord transected it was enough to bring them to slight conditions of asphyxia or to induce spinal facilitation by repeated application of the stimuli in order to obtain clear results (figures 4 and 7). As in the case of the intercostal and abdominal muscles, the phenomena of accommodation, recruitment, facilitation and summation could be obtained (figures 2, 4, 6 and 7).

The intensity of the applied stimuli needs not to be great, in many cases it was possible to see significant responses merely by touching the skin, particularly that at the thoracic regions, but some influence was also seen from the skin of the limbs or the tail.

All the reflex responses described in the respiratory muscles disappeared after section of the corresponding dorsal roots. In preparations in which a deafferentation was made of the thoracic segments, plus a section of the spinal cord at L 1, after double vagotomy, a stopping of rhythmic breathing was observed

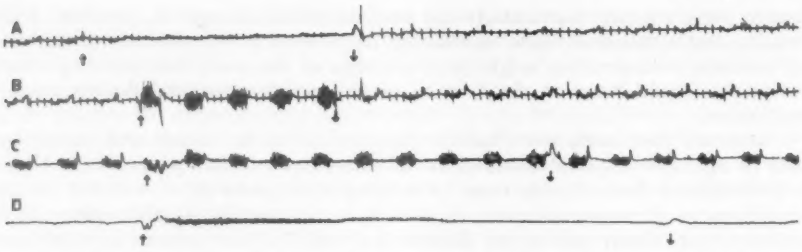


FIG. 4. — *Rhythmic activity of external intercostal muscles induced by the respiratory movements due to diaphragmatic contractions, and modified by superimposed afferent stimulation. Rabbit with the cord transected at T_4 and with double vagotomy. — A. – Lung inflation which reduced the thoracic wall movements. — B. – Lung deflation which enhanced the respiratory movements. — C. – Summation with the stretch reflex, tension was applied between arrows. Animal under light asphyxia due to increased dead space. — D. – The same maneuver as in C. Animal under hyperventilation.*

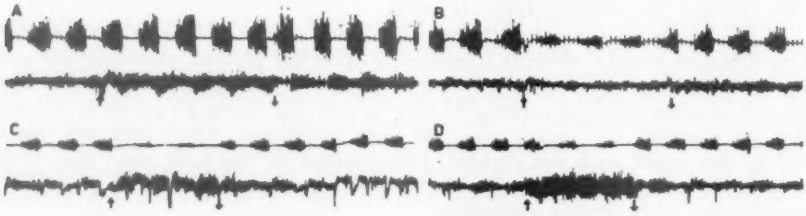


FIG. 5. — *Different and complex effects of afferent stimulation upon phrenic (upper records) and intercostal motoneurons (lower records). Rabbit with an incomplete section of the spinal cord at C_2, leaving intact the anterior funiculi. Double vagotomy. — A. – Between arrows, lung inflation (10 mm Hg). — B. – Results of lung deflation. — C. – Manual compression of the thoracic walls. — D. – Manual compression of the abdominal walls.*

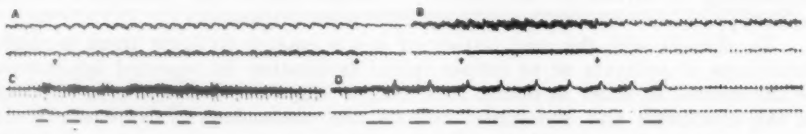


FIG. 6. — *Rhythmic displacement of the thoracic or the abdominal walls is enough to activate the motoneurons of respiratory muscles when they are under facilitation produced by repeated afferent stimulation. Rabbit, some time after transection of the spinal cord at C_2. Electromyograms of the diaphragm (above) and of external intercostal muscles (below). — A. – Recruitment under a continuous pressure applied upon the abdomen. — B. – Between signals, manual compression of the thoracic walls. With greater amplification in the electromyogram of the diaphragm in this and the following records. — C. – Rhythmic compression of the thoracic walls (horizontal lower lines). — D. – Rhythmic compression of the abdomen. In this case the residual inhibition is increasing every time.*

with continuous activity recorded from the diaphragm. This condition persisted as long as the animal was under artificial respiration and not hypoventilated. As soon as the stroke volume was reduced, rhythmic breathing could reappear again in relation to the degree of asphyxia.

Discussion

In the previous paper of this series ([3]) it was shown that expiratory activity shows responses to afferent stimulation. This influence does not merely appears as changes opposite in sign to those of the inspiratory muscles. This observation was taken to indicate that the concept of reciprocal inhibition of an inspiratory and an expiratory center was not compatible with those results, unless the afferent stimulation would exert its influence at the spinal level. It was also observed that the effects of vagal afferents, which also may give differential effects on expiratory muscles, could not influence the corresponding spinal motoneurons directly, and for that reason the hypothesis of reciprocal inhibition was rejected.

The present results, however, show clearly that the respiratory movements do exert some degree of control upon themselves at the spinal level, by way of proprioceptive or some other kind of somatic afferent impulses. There remains, thus, the possibility that afferent vagal stimulation could influence indirectly the responses of the respiratory muscles via the impulses elicited by the changes in position of the thoracic or abdominal walls. That this can happen is shown by the not unusual observation that the responses to inflation of the lung in intact animals may show, at the beginning or at the end of the depression in diaphragmatic activity, an enhancement of the muscle contractions.

With the exception of the deflation of the lung in animals with partially deafferented respiratory center (with an incomplete section at C 2, leaving the anterior funiculi intact), in which the response is a clear depression of diaphragmatic activity (figures 5 B and 7 B), most of the maneuvers upon the thoracic and abdominal walls evoke a positive response in all the respiratory muscles

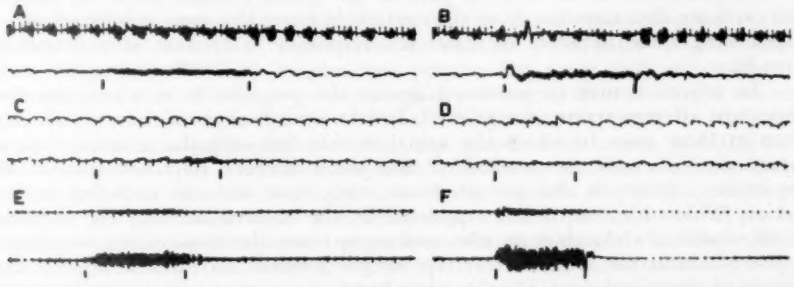


Fig. 7. — *Influence of the spinal section and of ventilation on the reflex responses of respiratory motoneurons. Rabbit with a section of the spinal cord at C2 which affected only the dorsal half of it. After C the cord was transected 2 mm above the previous section. Electromyograms of the diaphragm (above) and of external intercostal muscle (below). — A, C and E. – Between signals compression of the abdomen. — B, D and F. – Between signals compression of the thoracic walls. — A and B. – Before complete transection of the spinal cord. — C and D. – After transection, animal under artificial respiration. — E and F. – After stopping the artificial respiration.*

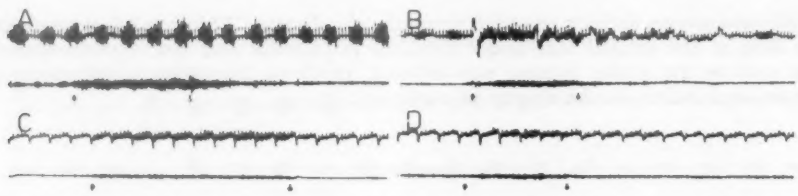


FIG. 8. — *The impulses from the respiratory center to the phrenic motoneurons have an inhibitory influence upon the reflex responses of those motoneurons to afferent stimulation. Rabbit with incomplete section of the spinal cord at C2. Electromyograms of the diaphragm (above) and of abdominal muscles (below). Between arrows, manual compression of the abdomen. — A. – Spontaneously breathing animal. — B. – Animal under artificial respiration, light hyperventilation. — C. – Animal moderately hyperventilated. — D. – After complete section of the spinal cord. Ventilation as in C.*

explored. It is true that inhibition currently outlasts the period of stimulation (figures 3 A, 4 A and 6 D). The question is how this kind of somatic afferent stimulation could interfere with the normal respiratory movements. It is interesting to note that most of the responses obtained are opposite in sign to the normal muscle activity during respiratory movements. This effect would tend to smooth respiration in the normal animal.

The threshold for the responses is relatively low. The normal respiratory movements, or those produced by the artificial respiration pump are usually enough to act as stimuli (figures 1 to 4, and figure 8). This, and the lack of spontaneous breathing after deafferentation of the thoracic spinal cord, with transection below this level and double vagotomy, points to the conclusion that respiratory movements exert a great influence on the normal integration of rhythmic breathing.

Inhibition occurred in most cases as part of the responses. It seemed more important when the stimulation might have induced a painful sensation. It appeared more sensitive to asphyxia. If the results on spinal animals are evidence for this phenomenon to occur as part of the spinal reflexes involved, there is also evidence that impulses from the centers do exert also some inhibitory effect. Suppressing these impulses increases the responses to afferent stimulation (figure 8).

An objection may be presented against the preparation in which the most important afferent tracts were severed. In fact, not all of them were interrupted. Even in those cases in which the spinal section left only the anterior funiculi intact, there is still the possibility that some afferent impulses reached the respiratory center via the spinothalamic tract, that was not included in that section. That objection is not supported by the observation that the responses of intercostal or abdominal muscles, and many times also those of the diaphragm, appeared about the same in this type of preparation and after complete transection of the spinal cord. On the other hand, the ventral spinothalamic tract is said to conduct tactile sensations (7), and most of the observed results were not obtained by this kind of stimulus. In most instances the displacement of the thoracic or the abdominal walls was necessary to produce the effects.

The responses to compression of the abdominal walls deserve some comment. They were obtained by applying pressure on any point of the belly, as well as by introducing air in the peritoneal cavity. The effective stimuli thus seems

to be the increase in pressure. The response did not come from stimulation of cutaneous receptors, as a fold of skin could be compressed between the fingers without inducing that response. Also, the responses do not depend from transmitted movements to the thoracic walls, since compression with a rod at the lower part of the belly gave a response as great as with the same maneuver applied at the upper places.

Summary

Reflex activation and inhibition of the motoneurons of the respiratory muscles is described on rabbits and cats under light anesthesia. A preparation was used in which there was a partial deafferentation of the respiratory center by an incomplete section of the spinal cord at C 2, leaving intact only the anterior funiculi. The results obtained with this preparation were fundamentally similar to those observed after complete transection of the spinal cord.

By the use of complex types of stimulation, most of them including displacements of the thoracic or the abdominal walls, it is shown that the activity of the spinal motoneurons in relation with respiratory muscles can be profoundly influenced (figures 3 to 8). The stretch reflex of the intercostal and abdominal walls are described (figures 1 and 2). The influence on the phrenic motoneurons is also shown.

Considering that activation or inhibition of the spinal neurons of respiratory muscles by afferent stimulation is of a low threshold, and that spontaneous breathing may stop by deafferentation of the thoracic segments of the spinal cord, together with total section below those levels and double vagotomy, it is concluded that somatic afferent stimulation derived from the respiratory movements themselves has an important influence on the normal integration of breathing and play a role in the determination of its rhythmicity. This part of the integration of respiration occur mainly at a spinal level.

Resumen

Se estudió la activación e inhibición reflejas de las motoneuronas correspondientes a los músculos respiratorios en conejos y en gatos bajo anestesia ligera con pentobarbital. Se empleó una preparación en la cual se produjo la deaferentación parcial del centro respiratorio, por medio de una sección incompleta de la médula espinal a nivel de C 2, dejando solamente los cordones anteriores. Los resultados obtenidos con esta preparación fueron fundamentalmente semejantes a los observados después de la sección completa de la médula.

Empleando estímulos complejos, que en su mayoría implicaban el movimiento de las paredes torácica o abdominal, se mostró que la actividad de las motoneuronas respiratorias puede ser grandemente afectada por vía refleja (figuras 3 a 8). Se describe el reflejo miotático de los músculos intercostales y abdominales (figuras 1 y 2). Contrariamente a las observaciones de otros autores ([2]), se muestra también una influencia grande de la estimulación aferente sobre las motoneuronas del frénico.

Teniendo en cuenta que la activación o inhibición de las motoneuronas de los músculos respiratorios por influencia somática aferente es de umbral bajo, y que la respiración espontánea puede cesar en un animal por la deaferentación de los segmentos espinales torácicos, con sección de la médula espinal

por abajo de estos niveles y la sección de ambos vagos, se concluye que la estimulación aferente somática derivada de los movimientos respiratorios mismos tiene una influencia importante en la integración normal de la actividad respiratoria, y que tiene cierto papel en la determinación de la ritmicidad de los movimientos. Esta parte de la integración de los movimientos respiratorios ocurriría a nivel espinal.

References

(1) Breckenridge, C. G. and Hoff, H. E.: *Amer. J. Physiol.*, 1954, *178*, 521.
(2) Calma, I.: *J. Physiol.* (Lond.), 1952, *117*, 9.
(3) García Ramos, J.: *Acta physiol. lat-amer.*, 1959, *9*, 246.
(4) Larrabee, M. G. and Hodes, R.: *Amer. J. Physiol.*, 1948, *155*, 147.
(5) Pitts, R. F.: *J. Neurophysiol.*, 1942, *5*, 75.
(6) Pitts, R. F.: *Physiol. Rev.*, 1946, *26*, 609.
(7) Ranson, S. W. and Clarck, S. L.: *The anatomy of the nervous system. Its development and function.* W. B. Saunders Co. Philadelphia and London, 1959.
(8) Wang, H. W. and Wang, S. C.: *Ann. Rev. Physiol.*, 1959. *21*, 151.

THE PRESSURE IN THE DIFFERENT ZONES OF THE PERICARDIUM

J. L. Duomarco, C. E. Giambruno and A. Correa Durán

(Instituto de Patología, Facultad de Medicina, Montevideo, Uruguay)

The pressure within the pericardial cavity, which has been previously actualized by the injection of air or liquid, has been considered in the study of many problems of cardiovascular physiology [2, 3]. It may be of interest, to study also the pressure in the virtual pericardial cavity, that is, in the smallest possible space created between the two pericardial leaves. Of course, this study implies the possibility that the pressure can vary within the pericardial zones. The local pericardial pressure is intimately related with the pericardial tension, that is, the tangential force which tends to break the pericardial membrane. At each point of the pericardial sac, this tension (T) depends on the pericardial local pressure (P) and on the local radius of curvature (R) of the membrane, according to Laplace's formula: $T = PR/2$. It is understood that, in this case, the formula can not be used to obtain numerical values. This paper reports the results obtained in a first experimental approach to the pressure-tension problem in the different pericardial zones.

Methods

Experiments were performed on dogs, anesthetized with chloralose (0.1 gm/Kg), with open thorax and under artificial respiration. A record of the local pressure-tension phenomena was obtained in the following way: a fairly firm rubber tube, 5 mm i.d., is attached to the pericardial wall with two stitches, as shown in figure 1-*I*. One end is closed and the other one is connected to an electromanometer. The whole system is filled with air at atmospheric pressure. Figure 1-*II* shows that the elastic camera incorporated in the pericardiac membrane is deformed by the synergic action of the pericardial tension *(T)* and the underlying pericardial pressure (P). There is not a clear distinction between both forces. The actual pericardial pressure was recorded by the usual procedure, after injection of certain quantities of air or saline solution. Right intraventricular or pulmonary arterial pressure were recorded simultaneously with the pericardial pressure, by means of a catheter introduced through the jugular vein.

Received for publication, May 25th, 1959.

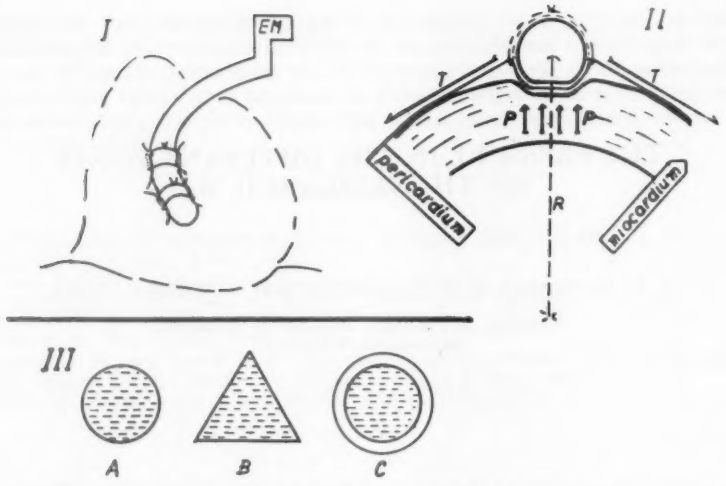


FIG. 1.—I) *Diagram of the experimental procedure. An elastic camera is incorporated to the pericardial membrane by means of two stitches.* II) *The elastic camera is deformed by the local pericardial pressure* (P) *and the pericardial traction* (T). R: *pericardial local radius of curvature.* III) *The diastolic volume of the heart* (A) *has a minimal surface of contact with the pericardium The same volume, by virtue of the systolic deformation* (B), *increases the pericardial surface of contact. This implies a greater potential pericardial volume, which has been actualized in* C. *See text.*

RESULTS

Figure 2 repeats a very well known experiment; it shows that the pressure waves of the distended pericardial cavity are inverted with respect to those of the right-ventricular pressure. This fact is easy to explain, because, during systole, almost all of the systolic volume leaves the heart through the arteries, while just a part of it reaches the heart through the veins. Within the terms of this experiment, pericardial waves increase with pericardial distension. Respiratory variations of the pressure in the pericardial cavity, when this is distended with saline, are due to corresponding vertical displacements of the heart.

Figure 3 reproduces three experimental sequences where pericardial pressure-tension is recorded on different ventricular zones and compared with right-ventricular or pulmonary artery pressure curves. During each sequence respiration is stopped in order to permit the action of asphyxial phenomena (slow rate, weakness of the heart beat, heart dilatation) on the local pressure-tension of the pericardium to be studied. Observation shows: a) Pericardial waves on the ventricular area are not inverted with respect to, but have the same direction as the intraventricular or arterial waves. b) The amplitude of the pericardial waves increases at the beginning, by virtue of heart dilatation, and decreases towards the end, when the heart beat becomes extremely weak. c) The pericardial waves show a clear retard with respect to ventricular systole. This retard increases when the ventricular activity becomes weaker. d) There is no retard between pericardial and pulmonary artery pressure curves.

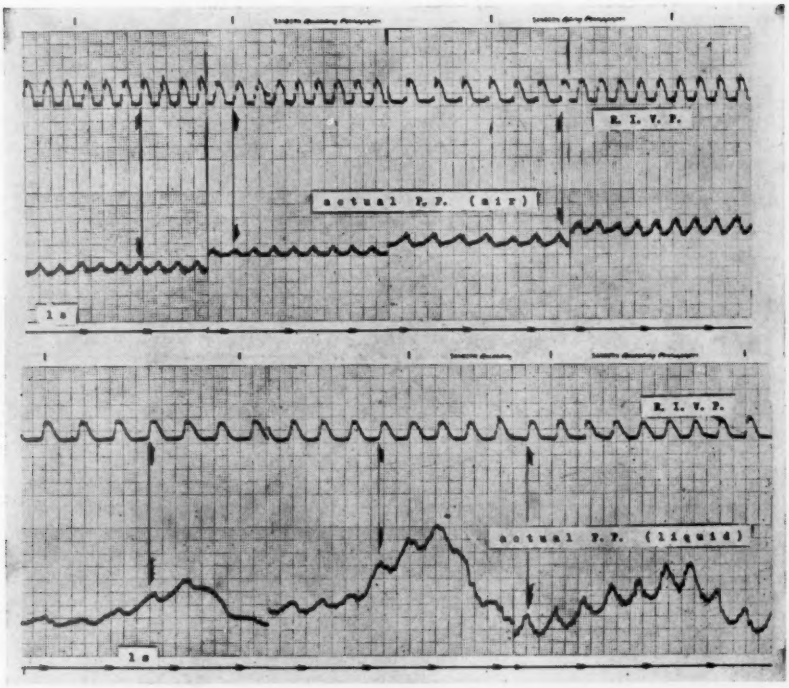


FIG. 2. — *Right intraventricular pressure (R.I.V.P.) and actual pericardial pressure (P.P.). Increasing pericardial distension.* Top: *Four stretches (with air).* Bottom: *Three stretches (with saline solution). The beginning of some waves is marked. See text.*

Figure 4 shows: a) Pericardial waves on the right-ventricular conus have the same direction as the right-ventricular pressure waves, and the expected retard. b) Pericardial waves on the right atrial zone are also positive and precede the ventricular waves: they evidently correspond to auricular systole. c) Pericardial waves on the left ventricle increase with the ventricular mechanical impulse (adrenalin action) and start simultaneously with the pulmonary artery pressure waves.

DISCUSSION

The paradoxical behaviour of the pericardial local waves, in comparison with the waves of the actual pericardial cavity, results from the fact that the latter belong to a true plethysmographic curve, while the former are a consequence of the ballistic recoil of the underlying heart zone against the pericardial wall, during the ejection period of the systole. This explains why these recoil waves increase with the systolic impulse (adrenalin) and with the local radius of curvature of the pericardium (asphyxial heart dilatation). It also supplies an expla-

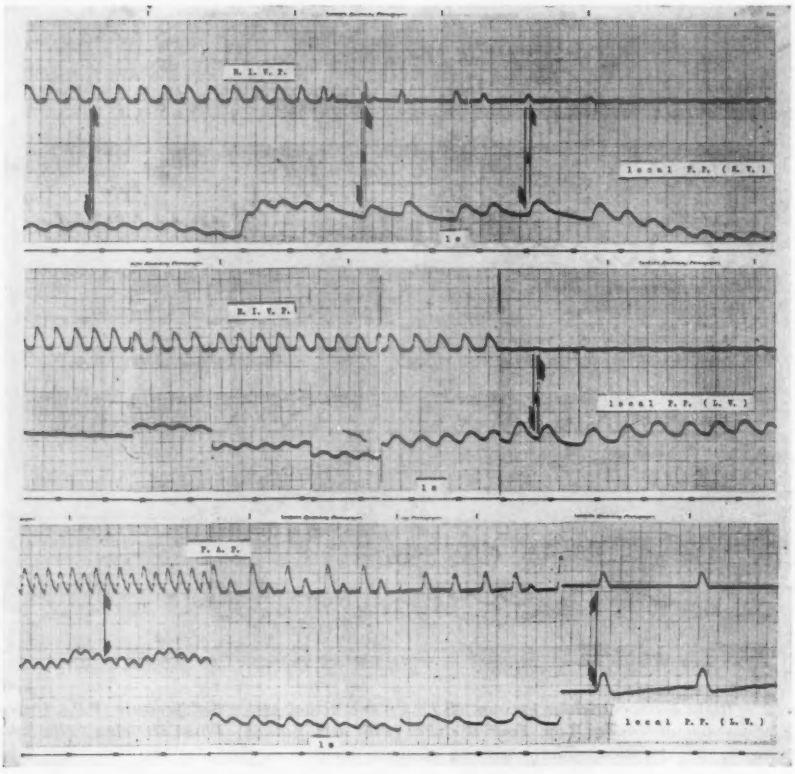


FIG. 3. — *Local pericardial pressure-tension (P.P.) uppon different cardiac areas* (R.V.: *right ventricle;* L.V.: *left ventricle) and right intraventricular pressure* (R.I.V.P.) *or pulmonary arterial pressure* (P.A.P.). *Three experimental sequences with progressive asphyxia. The beginning of some waves is marked. See text.*

nation for the fact that those pericardial waves are simultaneous with the ejection period and not with the whole systolic event. It is evident that the vectorial sum of the forces caused by the ballistic recoil of the different parts of the heart generates the cardiac component of the ballistocardiogram (4), and that the pericardium is the first mechanical intermediary between the heart and the mass of the body.

From another point of view, in normal and pathological conditions, the pericardial sac maintains a size and shape which is related with its contents. It is difficult to conceive of any modelling force other than the distension of the pericardial wall, due to the pressure exerted by the heart. This modelling action should be performed by two different mechanisms: *I*) *In diastole*, the heart practically constitutes an hydrostatic mass; consequently, its modelling function begins only when it has totally unfolded the pericardial cavity, and when the central

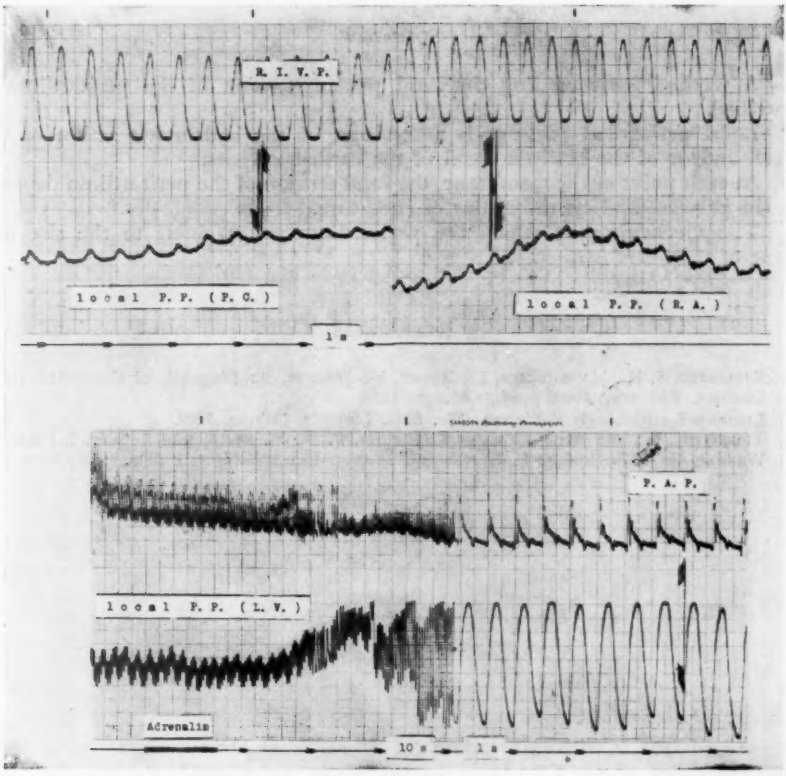


FIG. 4. — *Local pericardial pressure-tension* (P.P.) *on different cardiac areas* (P.C.: *pulmonary conus;* R.A.: *right atrium;* L.V.: *left ventricle) and right intraventricular or pulmonary arterial pressure. The beginning of some waves is marked.* Bottom: *injection of adrenalin; change of velocity. See text.*

venous pressure begins to increase. Obviously, this action affects the size but not the shape of the pericardium. *II*) *In systole*, the ballistic recoil of the heart cavities exerts the main modelling action, which affects size and shape of the pericardium. Particularly, this mechanism can increase the *potential* capacity of the pericardium over the maximal diastolic volume. In this respect, diagrams in figure 1-III attempt to represent: (*A*) End-diastolic volume and cardio-pericardial surface of contact (mechanism *I*). (*B*) Early systolic deformation that increases surface of contact and potential capacity of the pericardium (mechanism *II*). (*C*) Potential pericardial capacity made real by an injection of fluid.

The *apex beat* and the relative inmovability of the apex, thoroughly studied by the classics ([2, 3, 4]), is partly related to the ballistic recoil of the ventricles against the thoracic wall, in systole. Modern angiocardiographic studies ([1]) show relative inmovability of atrial portions opposed to the atrioventricular valves during auricular systole.

Summary

A method for recording the local pressure-tension of the pericardium is described.

Local pericardial pressure is a resultant of the hydrostatic action of the heart, and/or of the ballistic recoil of the cardiac cavities.

At each point of the membrane, the local tension of the pericardium depends on the pressure and on the radius of curvature.

The mechanism by which the pericardium adapts itself to the size and shape of the heart is discussed.

References

(1) Kjellberg, S. R., Mannheimer, E., Rudhe, U., Jonsson, B.: Diagnosis of Congenital Heart Diseases. *The year Book publ.*, Chicago, 1954.

(2) Luciani, L.: Fisiologia dell'uomo. *Soc. Edit. Libraria*, Milano, 1920.

(3) Tigerstedt, R.: Die Physiologie des Kreislaufes. *V. W. V.*, Berlin und Leipzig, I, 1921.

(4) Wiggers, C. J.: *Physiology in Health and Disease.* Lea & Febiger, Philadelphia, 1944

FUNCTIONAL MODIFICATIONS OF THE PACEMAKER AND CONDUCTING SYSTEMS OF THE HEART IN EXPERIMENTAL HYPER-AND HYPOTHYROIDISM

H. Van Den Beld C., R. Douglas R. and J. Talesnik D.

(Department of Physiopathology, University of Chile, Medical School, Casilla 6510, Santiago de Chile)

It is well known that patients with functional disorders of the thyroid gland show definite disturbances of the cardiac rhythm. Thus, in the hyperthyroid state the heart rate is increased. Physical exertion or emotional excitement may increase this rapid rate even more. Tachycardia is maintained even during sleep, in contrast to what happens in other conditions associated with increased heart rate such as in certain psychoneurotic states ([8]).

It should be pointed out that the cardiac rhythm in hyperthyroidism is disturbed by extrasystoles, frequently of ventricular origin. In many cases of long standing hyperthyroidism, atrial flutter or fibrillation is present ([3, 6, 11, 29, 30, 31, 37]). On the contrary, in myxedematous states that are usually accompanied by bradycardia, extrasystoles are rarely observed, except in cases of circulatory disorders of the heart derived from coronary atherosclerosis ([5]).

In order to explain the mechanism involved in the development of tachycardia in hyperthyroidism, compensatory factors due to increased metabolic requirements were first considered ([8, 30]). When this explanation was suggested no reference was made to the fact that in thyrotoxicosis the oxygen arterio-venous difference is reduced although there is marked increase in metabolic rate ([7, 22]). The reduction in oxygen arterio-venous difference could be due to an accelerated blood flow, since it has been shown that in hyperthyroidism there is an

Received for publication, June 15th, 1959.

increased heart rate with hypervolemia ([9]) and consequently increased cardiac output. According to these facts the augmented heart rate in hyperthyroidism could be explained as a reflex mechanism of the Bainbridge type, initiated by the exaggeration of the venous return ([8]). Nevertheless, there are no conclusive experiments showing that this reflex mechanism is responsible for the acceleration of the heart in thyrotoxicosis.

The mechanisms involved in the increased frequency of the heart that occurs in hyperthyroidism are different from those inducing changes in cardiac rate in fever or dinitrophenol intoxication. For instance when the metabolic rate is increased by dinitrophenol the heart rate is slightly changed. On the contrary, when metabolic alterations are induced by lack or excess of thyroid hormone, marked modifications of cardiac rhythm are obtained ([20]). Besides, there is experimental evidence showing that the thyroid hormone has an influence on the basic heart rate by a direct action upon the myocardium.

In fact, changes of cardiac rate determined by lack or excess of thyroid hormone are present even in isolated hearts ([12, 17, 21, 28, 38]). This effect of the thyroid hormone concerns the excitability of the pacemaker and conducting systems among other actions, as suggested by experiments performed on chick embryo tissue culture ([23]).

In dogs with total cardiac denervation, tachycardia has been induced as well as in animals with intact nerve supply to the heart ([24]). Further studies on denervated hearts of hyper and hypothyroid animals tend to show that tachycardia and bradychardia respectively, are independent of the nervous system ([25]). Nevertheless these experiments do not exclude the influence of the nervous system on a substrate already modified by the endocrine disorder.

Clear differences were found when the action of the cardiac nerves and the neurotransmitter substances were studied on isolated hearts of hyper and hypothyroid animals. This, and the fact that changes in frequency persist in isolated hearts ([14]), support the hypothesis that postulates a cardiogenic mechanism of rhythm alterations under thyroid influence ([12, 17, 21, 28]).

In the experiments described below it was found that the thyroid hormone has a marked influence on the pacemaker and conducting system of the heart that could explain the origin of the disturbances of the cardiac rhythm. The experiments were performed in isolated mammalian heart with sections at different levels of the specialized system. Pharmacological blockade was also induced with acetylcholine which has been shown to establish rhythms of infrasinusal origin ([26, 32]).

Methods

Experiments in rats.

Seventy five animals, of mixed breeding whose body weight ranged from 170 to 320 g were studied. They were given food and water "ad libitum". The oxygen consumption was measured every other day according to the procedure described elsewhere ([27]). After two or three weeks, the animals were selected for the following groups:

a) Seventeen rats were injected daily with thyroxine 1 mg/100 g body weight subcutaneously. The treatment was followed for 5 to 12 days.

b) Four rats, after 6 to 9 days of treatment with thyroxine, were allowed to recover without any treatment for 27 to 43 days (to be called, post-hyperthyroid).

c) Thirty "normal animals" which were considered as controls.

d) Twenty-four rats in which thyroidectomy had been carried out 11 to 49 days prior to the experiment.

At the times indicated, the rats were anesthetized with pentobarbital (intraperitoneal injection of 2.5-5 mg/100 g body weight) and electrocardiographic recording of heart rate was obtained. The preparation of Langendorff was then performed. Tyrode solution at 38° C was perfused at 45 mm Hg. The ventricular activity was recorded with an isotonic lever on smoked paper.

Experiments in cats.

Cats of mixed breeding whose initial body weight ranged from 1000 to 3100 g were grouped in the following way:

1) *Thyreotoxicosis.* Ten animals were injected subcutaneously with thyroxine, 1 mg/Kg body weight, for 21 to 31 days.

2) *Hypothyroidism.* Eight cats were thyroidectomized 25 to 35 days before the experiments.

3) *Normals.* Ten animals taken at random were considered as controls.

As in the rat experiments, the hearts were perfused according to the Langendorff method.

Results were compared with Student's "t" test.

Results

Experiments in rats.

The experiments in which elimination of the sinus-auricular node and section of the atrio-ventricular bundle were performed, will be referred to first.

The heart rate obtained in the anaesthetized animal by electrocardiogram will be denominated ECG rate, while the frequency of the isolated heart with a normal rhythm will be referred as SR (Sinus Rhythm); NR (Nodal Rhythm) will denote the rhythm obtained after both auricles were sectioned and IVR (Idio Ventricular Rhythm) the heart rate that appears after crushing the upper region of the interventricular septum.

In figures 1 to 6 the heart rate is plotted in relation to the O_2 consumption measured just before the experiment was carried out. As it can be seen in figure 1, the ECG values of the hypo and hyperthyroid rats are significantly separated from the normals. The higher the O_2 consumption, the higher the ECG and the opposite can be said for the hypothyroid rats. It should be emphasized however that the group of animals treated with thyroxine and allowed to recover from the treatment, showed a return of O_2 consumption to normal levels, but nevertheless, the cardiac rate remained of the same magnitude as in those rats with increased metabolic rate.

In figure 2, it can be seen that the differences for the SR values show the same trend as the ECG values in all groups.

When the auricles were excluded, the heart rate dropped in all groups, but the values for the post-hyperthyroid animals no longer differed from the normals. It can also be seeen in Fig. 3, that the NR of the hyperthyroid rats was higher than the post-hyperthyroid, normal and hypothyroid animals. There is no significant difference between the post-hyperthyroid and the normal, but both these groups show faster NR than hypothyroid rats.

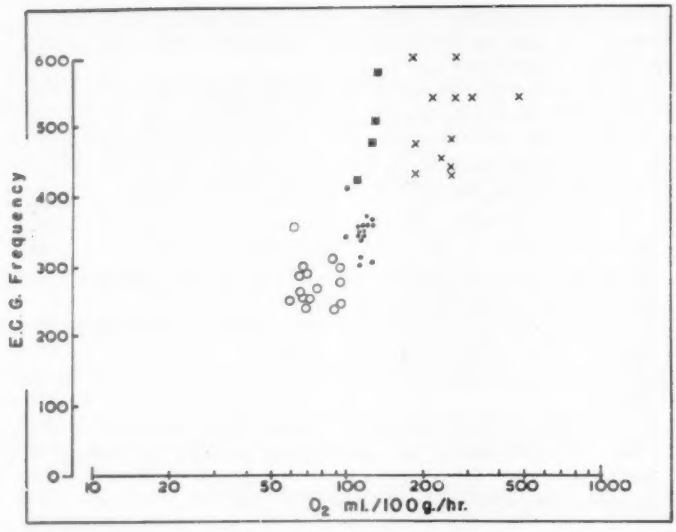


FIG. 1.—*Heart rate measured "in situ" by E.C.G. plotted against O_2 consumption.*
× *hyperthyroid*
⊠ *post-hyperthyroid*
• *normal*
○ *hypothyroid*

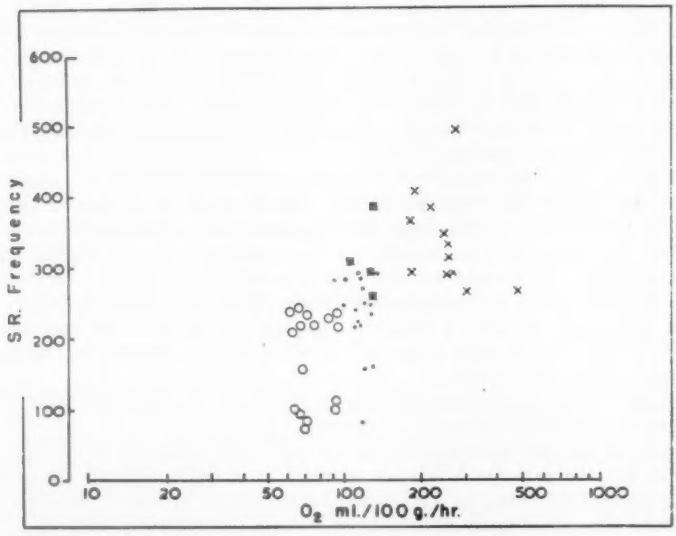


FIG. 2.—*Heart rate in isolated perfused hearts with sinus rhythm plotted against O_2 consumption* (see text). Symbols as in Fig. 1.

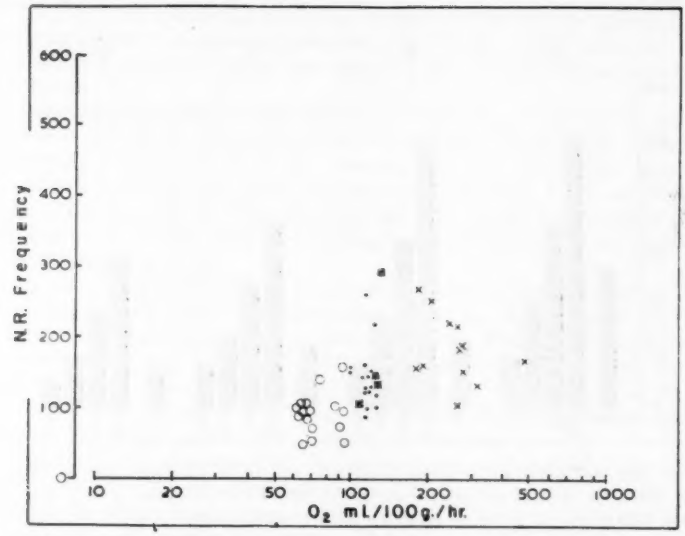


FIG. 3.—*Heart rate in isolated perfused hearts with auricles excluded plotted against O_2 consumption* (see text). Symbols as in Fig. 1.

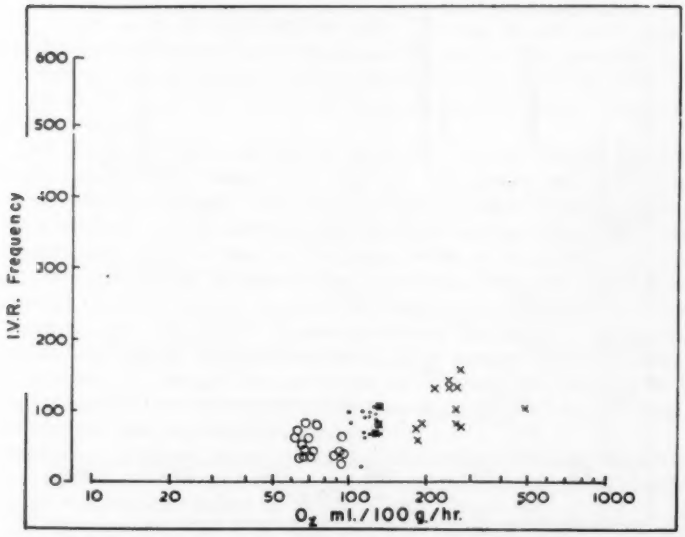


FIG. 4.—*Heart rate in isolated perfused hearts, after interventricular clamping, plotted against O_2 consumption.* Symbols as in Fig. 1.

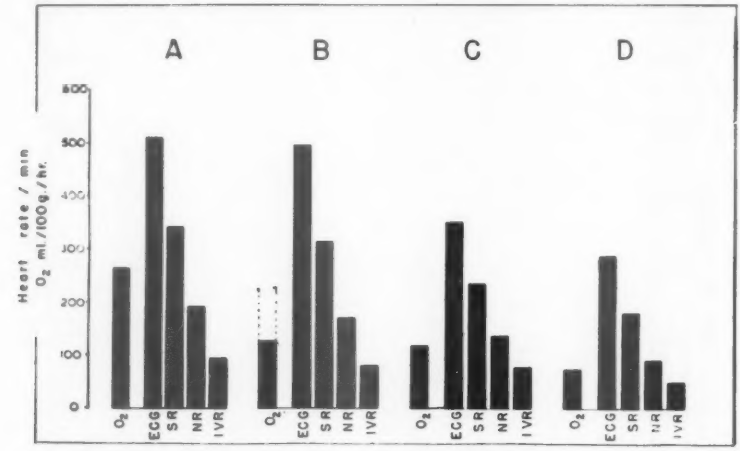


FIG. 5.—*Comparative study of the mean heart rate and O_2 consumption in:* A: *Hyperthyroid group;* B: *post-hyperthyroid group;* C: *normal group;* D: *hypothyroid group. Discontinuous lines in the O_2 column of group* B *show the highest level reached during the treatment with thyroxine. O_2 = Oxygen consumption;* E.C.G. = *Heart rate measured "in situ" by electrocardiogram;* S.R. = *Sinus rhythm in isolated hearts;* N.R. = *Nodal rhythm in isolated hearts;* I.V.R. = *Idioventricular rhythm in isolated hearts.* (See text.)

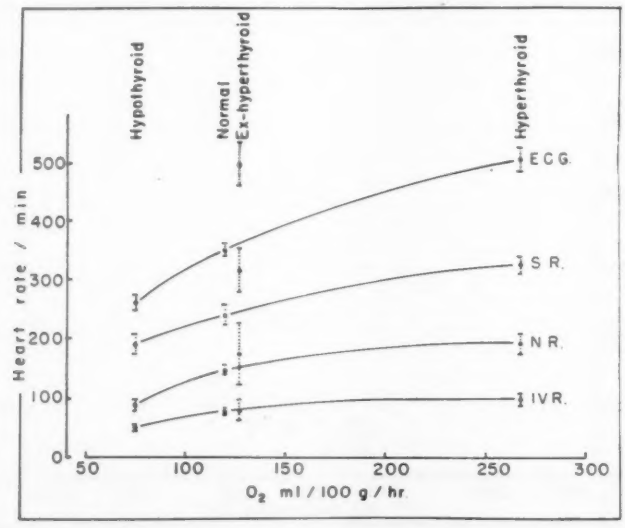


FIG. 6.—*Mean values (and standard deviation of the mean) of heart rate plotted against mean oxygen consumption. Symbols as in Fig. 5.*

In Fig. 4 the values for IVR are plotted and as can be appreciated the IVR of thyrotoxic rats are superimposed on the IVR of normal animals. Nevertheless there are still statistical differences between the rate of IVR of hyperthyroid and hypothyroid rats.

Before entering into further details, it is convenient to look at the experiment as a whole helped by figures 5 and 6. It is evident that there is only an apparent relationship between level of O_2 consumption and ECG rate, since the hearts of post-hyperthyroid rats that had recovered normal levels of metabolic rate behave like those of the thyroxine treated rats; on the other hand SR, NR and IVR from the post-hyperthyroid group are indistinguishable from the values of the hyperthyroid one.

In all groups, the sinus frequency of the perfused heart is lower than the corresponding heart rate measured by ECG. As a matter of fact the SR in the hyperthyroid group diminished by 35,5 %, while in the post-hyperthyroid, the diminution was 33 %; in normal and hypothyroid groups the decreased frequency was about 33 and 36 % respectively. The reduction in heart rate due to lower pacemakers is of the same degree in the different groups if one considers the ECG as the basic rate for comparison.

Another way of comparing the characteristic rhythms in the different conditions studied can be appreciated in fig. 6, where the average values and standard deviation of the mean are shown. It is evident that the general trend is kept for each rhythm by groups of hyperthyroid, normal and hypothyroid rats. While the greater differences appear for ECG and SR rhythms, the differences are less for the lower NR and IVR.

Once more it can be easily seen that the mean values of heart rate of the post-hyperthyroid group, that had an O_2 consumption in the range of the normal ones, are similar to those of the hyperthyroid group.

In a new series of experiments only 3 groups of rats were employed. The group allowed to recover from thyroxine treatment, was omitted. Acetylcholine was added to the perfusion fluid in concentration of 1.5×10^{-6} and a quick injection of 10 μg of the drug reinforced the action about 2 minutes after the perfusion started.

The ECG rhythm was higher than the SR as already described in the previous experiments. When acetylcholine was given the heart beat stopped and a new rhythm started. This new rhythm is called "rhythm of escape" (ER).

It is evident that the acetylcholine-induced cardiac arrest lasted much longer in hypothyroid animals than in normals, while in hyperthyroid rats the heart arrest was significantly reduced when compared with the other two, as it can be seen in figures 7 and 8. In fact the heart stopped longer when the animal O_2 consumption was lower (hypothyroidism) and shorter when the O_2 consumption was higher (hyperthyroidism). The longer cardiac inhibition lasted, the slower was the escape rhythm of the heart as can be observed in fig. 9. The average values of ER of hyperthyroid rats were slightly higher than the mean of the normal and hypothyroid groups.

Statistical analysis shows that the difference between hyperthyroid and normal rate of heart escape is not significant, due to the small number of cases and a great spread of values in the hyperthyroid group.

If one compares the IVR and the ER in fig. 6 and 9, the similarity of both curves is striking suggesting a certain relationship in the origin of the pacemaker in either condition.

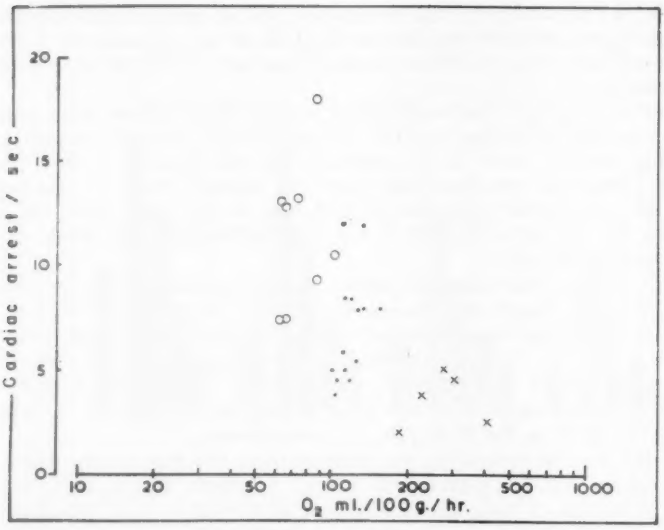


FIG. 7. — *Duration of cardiac arrest induced by acetylcholine plotted against oxygen consumption.*
○ = hypothyroid
• = *normal*
× = *hyperthyroid*

On the other hand, fig. 9 shows that a certain inverse relationship can be established between original rate (ECG or SR) and the length of the standstill.

Experiments in cats.

Similar conclusions were drawn from experiments performed on isolated perfused cat's heart, with the advantage that the different rhythms obtained were checked electrocardiographically.

No statistical study is shown because the spread of values in the small number of cases was more noticeable. Nevertheless it can be said that comparison of individual experiments of long treated cats with the normal ones show typical differences that can be observed in figure 10.

In the records of figure 10 the difference in SR and rhythm of escape induced by acetylcholine perfusion can easily be appreciated.

It is known that when an infranodal rhythm is present the depressor action of acetylcholine is ineffective ([26]). To make sure that the rhythm obtained during perfusion with acetylcholine was of infranodal command, high doses of acetylcholine were quickly added to the heart and no alterations of the mechanical or electrographic recording were seen.

The absence of P wave with acetylcholine corresponded to arrest of the activity of the auricles registered on the kymograph.

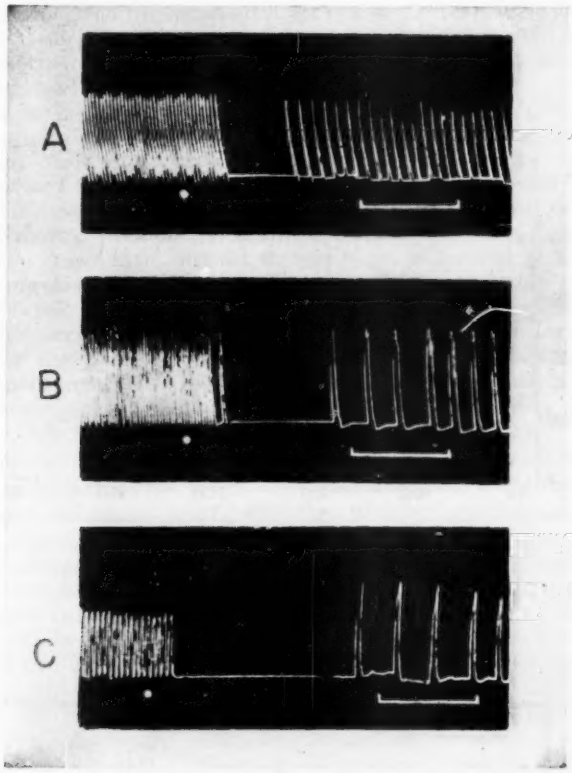


FIG. 8. — *Heart standstill and escape after acetylcholine in* A: *hyperthyroid rat.* B: *normal rat;* C: *hypothyroid rat. Dots indicate injections of acetylcholine. Time scale 5 sec.*

DISCUSSION

The described experimental results emphasize that the modifications of basic heart rate are mainly due to a direct action of the thyroid hormone on the heart. The demonstration of the direct action of the thyroid on the cardiac muscle is unquestionable since changes in frequency of the cardiac rhythm obtained in the intact animal by thyroidectomy or when thyroxine is given, persist in the isolated perfused heart ([17, 21, 38]).

The influence of the thyroid was further emphasized in those few cases where thyroxine was given to rats and after a while the treatment stopped although it should be confirmed with a larger number of experiments. In these animals the O_2 consumption dropped down to normal levels, nevertheless tachycardia persisted at about the same magnitude as in rats continuously treated with thyroxine.

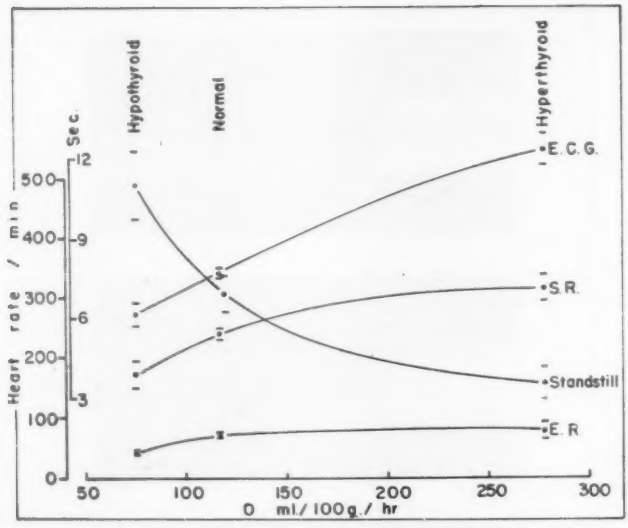


FIG. 9. — *Comparative study of the mean values and standard deviation of the heart rate measured "in situ" (ECG), in the isolated preparation* (SR), *the "escape rhythm"* (ER) *and the standstill obtained with acetylcholine.*

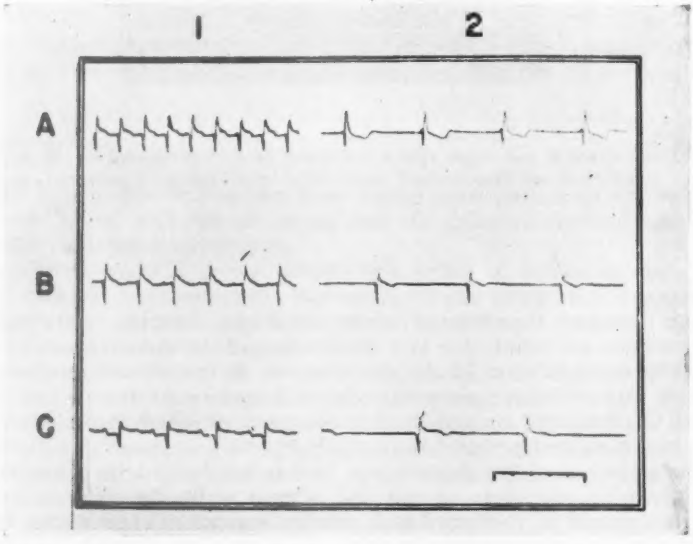


FIG. 10. — *Electrographic records from isolated cats'hearts.* A: *hyperthyroid;* B: *normal;* C: *thyroidectomized;* Column 1: *sinus rhythm;* Column 2: *rhythm of escape. Time scale 1 sec.*

It is interesting that the increased heart rate in the thyrotoxic state continues after the metabolic rate has reached normal levels. This observation points towards a dissociation between the direct action of the thyroid hormone on the effector systems and the level of increased calorigenesis ([20]).

It would not be correct to assume that the action of the thyroid hormone has only a pathologic influence on the heart. Probably the actual basic frequency of the normal heart is partially due to the influence of the thyroid gland and the decreased heart rate in hypothyroidism and increased frequency in hyperthyroidism should be only considered as an exaggeration of the normal action of the thyroid hormone on the myocardium.

It has been shown then, that the thyroid gland influences the heart function through two mechanisms: one concerns the speed of contraction of the muscle itself ([13, 19, 36]) and the other affects the cardiac rhythm. In experimental thyrotoxicosis the speed of the mechanogram is increased for the skeletal muscle as well as for the heart muscle ([1, 15, 16]). Furthermore, it has been reported that in hearts from hyperthyroid animals, cooling of the sinus node decreases the rate without parallel reduction of the speed of contraction as in normal cases ([18, 36]).

In this paper, proof is given showing that the thyroid hormone acts specifically on the pacemaker system When a new pacemaker was established, differences in the rate were maintained among the hearts of hyper, normal and hypothyroid animals. On the other hand, the analysis of the results shows that the influence of the thyroid hormone is greater in the upper regions of the pacemaker system since the difference of cardiac rate when the pacemaker is lower are less marked among different groups.

This could be the explanation for the observations in human subjects with A-V block in which hyperthyroidism was induced: only the auricles showed rhythm alterations while no significant changes occurred in the ventricles ([2]).

It could be inferred from these experiments, that the thyroid hormone modifies the pacemaker and conducting systems in its property of generating impulses. Support for this assumption was obtained from experiments where the time of heart arrest was measured after exclusion of the sinus-auricular node. In fact, in thyroxine-treated animals, the heart arrest was shorter and accordingly with the hypothesis, it would mean that infrasinusal regions have a more developed capacity to take over the cardiac pacemaker. The opposite was clearly demonstrated when shift of the pacemaker was obtained in hearts of thyroidectomized animals.

Furthermore it could well be that many disturbances of the heart rhythm in hyperthyroidism like tachycardia, ventricular extrasystoles, flutter or auricular fibrillation could be related to the high capacity to produce ectopic impulses in the heart in relation with changes of the metabolic activity demonstrated for the thyroid hormone on the heart ([10, 33, 34]).

It has not been clearly demonstrated how the pacemaker is established, but some relationship with the ability to synthesize acetylcholine and heart automatism has been found ([4]). On the other hand, acetylcholine synthesis can be interfered with through different influences. Among others, it has been shown in nervous tissue that the speed of acetylcholine synthesis is increased in conditions of hyperthyroidism ([35]).

It would be hazardous from this indirect evidence to draw conclusions about the mechanism by which the thyroid hormone influences the automatic

activity of the heart. Anyhow it would be worth while to consider the relation between synthesis of acetylcholine by the heart and the action of the thyroid hormone on the spontaneous activity of the pacemaker and conducting systems.

Summary

Previous reports have shown that the thyroid hormone affects the cardiac rate by a direct action on the heart.

The present work supplies more evidence that hearts of hyper and hypothyroid rats and cats maintain a characteristic tachycardia and bradycardia when isolated. Studying the mechanism by which the thyroid hormone modifies the heart rate, it was found that:

1) The sinus rhythm is significantly different in the hearts of the three groups studied, namely hypothyroid, normal and hyperthyroid animals.

2) The removal of the sinus pacemaker leads to a decreased heart rate, but the differences observed in the three experimental groups are maintained.

3) If the interventricular septum is sectioned, the heart rate decreases even more, yet the differences between ventricular rate of hypo- and hyperthyroid animals persist.

4) Cardiac escape of infranodal origin develops by blocking the auricular pacemaker with acetylcholine. The ventricular rhythm thus obtained in the three groups has the same characteristics as the one described above.

5) The influence of thyroid hormone on the various segments of the pacemaker and conducting systems is evident when a new pacemaker is established. Nevertheless, the influence is greater in the upper regions of the pacemaker system since the difference of cardiac rate when the pacemaker is lower is less marked.

6) The cardiac arrest that precedes the idioventricular rhythm, is significantly greater in hearts from hypothyroid animals than in normal and hyperthyroid ones.

7) Thyroxine-induced tachycardia persists long after hormonal treatment is discontinued, although oxygen consumption has returned to its normal level.

These observations emphasize the importance of the thyroid hormone as one of the factors with a regulating action on the genesis of impulses that determine the basic cardiac rate.

These findings and their possible relations to some cardiac disturbances frequently observed in myxoedema and thyrotoxicosis are discussed.

We acknowledge the help of Dr. G. Hodgson in revising the manuscript.

References

(1) Ananías, S., Hoffmann, F., Hoffmann, E. J. y Markovich, S.: *Bol. Soc. Biol.* Stgo. Chile, 1948, *6*, 34.
(2) Aub, J. C. and Stern, N. S.: *Arch. intern. Med.*, 1918, *21*, 130.
(3) Blumgart, H. L. and Stone, A. F.: *Circulation*, 1952, *6*, 222.
(4) Bülbring, E. and Burn, J. H.: *J. Physiol.* (Lond.), 1949, *108*, 508.
(5) Ellis, L. B., Mebane, G. J., Maresh, G., Hultgren, H. N. and Bloomfield, R. A.: *Amer. Heart J.*, 1952, *43*, 341.
(6) Ernstane, A. C.: *Amer. J. med. Sci.*, 1938, *195*, 248.

(7) EWIG, N. und HINSBERG, K.: ct. s (8).
(8) FRIEDBERG, C. K.: *Diseases of the Heart*, 2nd. ed., Philadelphia, W. B. Saunders Co., 1956.
(9) GIBSON, J. C. and HARRIS, A. W.: *J. clin. Invest.*, 1939, *18*, 59.
(10) GOH, K. O. and DALLAM, R. D.: *Amer. J. Physiol.*, 1957, *188*, 514.
(11) GRINSWOLD, D. and KEATING, J. H.: *Amer. Heart. J.*, 1949, *38*, 813.
(12) HIRVONEN, L. and LYBECK, H.: *Acta physiol. scand.*, 1956, *36*, 23.
(13) HIRVONEN, L. and LYBECK, H.: *Acta physiol. scand.*, 1956, *36*, 29.
(14) HOFFMANN, F., HOFFMANN, E. J. and TALESNIK, J.: *Amer. J. Physiol.*, 1947, *148*, 689.
(15) HOFFMANN, F., HOFFMANN, E. J. y MARKOVICH, S.: *Bol. Soc. Biol.* Stgo. Chile, 1948, *3*, 44.
(16) HOFFMANN, F. and HOLMGREN, B.: *Acta physiol. lat.-amer.*, 1953, *3*, 25.
(17) HOFFMANN, F., MIDDLETON, S., MOLINA, A. and TALESNIK, J.: *Acta physiol. lat.-amer.*, 1953, *3*, 118.
(18) HOFFMANN, F., MIDDLETON, S. y MANHOOD, M.: *Bol. Soc. Biol.* Stgo. Chile, 1953, *10*, 20.
(19) KARASEK, F. et KRUTA, V.: *C. R. Soc. Biol.*, París, 1937, *126*, 121.
(20) LEBLOND, C. P. and HOFF, H. F.: *Amer. J. Physiol.*, 1944, *141*, 33.
(21) LEWIS, J. K. and MCEACHERN, D.: *Bull. Johns Hopk. Hosp.*, 1931, *48*, 226.
(22) LILJESTRAND, G. and STENSTRÖM, N.: ct. s (8).
(23) MARKOWITZ, C. and YATER, W. M.: *Amer. J. Physiol.*, 1932, *100*, 162.
(24) MCINTYRE, M.: *Amer. J. Physiol.*, 1932, *99*, 261.
(25) MCKAY SAWYER, M. S. and BROWN, M. G.: *Amer. J. Physiol.*, 1933, *100*, 520.
(26) MIDDLETON, S., MARTNER, J. y TALESNIK, J.: *Bol. Soc. Biol.* Stgo. Chile, 1951, *8*, 145.
(27) MUÑOZ, O. E.: Acción de la tiroxina sobre la cuantía del metabolismo en ratas sometidas a dietas ricas en grasa y relativamente pobres en colina. *Tesis Inst. Fisiol. U. Chile.* (1944).
(28) PRIESLEY, J. T., MARKOWITZ, J. and MANN, F. C.: *Amer. J. Physiol.*, 1931, *96*, 348.
(29) RACHMILOWITZ, M. and COHEN, A. M.: *Cardiología*, 1952, *21*, 29.
(30) SPENCE, A. W.: *Clinical Endocrinology*, London, Cassel & Co. Limt., 1953.
(31) STAMBURY, J. B.: *Mod. Concepts Card. Disease*, 1952, *21*, 134.
(32) TALESNIK, J., HOFFMANN, F., MIDDLETON, S. and MARTNER, J.: *Proc. XIX Internat. Physiol. Congress*, 1953, p. 819.
(33) THOMAS, H. M.: *Arch. intern. Med.*, 1957, *99*, 165.
(34) ULLRICK, W. C. and WHITEHORN, W. V.: *Amer. J. Physiol.*, 1952, *171*, 407.
(35) VALLEJOS, F., BENÍTEZ, D., MIDDLETON, S. y TALESNIK, J.: *Bol. Soc. Biol.* Stgo. Chile, 1950, *8*, 66.
(36) VOLOSKY, R.: Acción de la hormona tiroidea sobre la velocidad de contracción cardíaca. *Tesis Lic. Fac. Med. U. Chile.* (1948).
(37) WAYNE, E. J.: *Guy's Hosp. Gazette*, 1952, *66*, Nº 1630:85.
(38) YATER, W. M.: *Amer. J. Physiol.*, 1931, *98*, 338.

INDUCTION OF ARGINASE SYNTHESIS IN AN AUXOTROPH OF NEUROSPORA CRASSA

JULIO CABELLO, RUTH URBÁ, VICTORIA PRAJOUX and CARLOS BASILIO

(Instituto de Química Fisiológica y Patológica de la Escuela de Medicina, Universidad de Chile, Santiago, Chile.)

IN 1944, Srb and Horowitz (1) reported that arginase is present in mycelia of wild type and arginine-less mutant strains of *Neurospora crassa*. Wild type strain cultivated in minimal medium enriched with arginine, was shown to have two or three times more arginase activity than cells grown in the unsupplemented medium. These authors termed the enzyme as "partially adaptive".

Here we report studies on the synthesis of arginase by an arginine-less mutant of *Neurospora crassa*. The absolute and specific dependence of the auxotroph development on an exogenous source of arginine may implicate the arginase activity of this mutant as an exacting mechanism for starting the cell growth. On account of this circumstance, the rate of arginase production in this mutant may be conspicuously different from the wild type. The effects of the amount of arginine in the medium, the supply of other aminoacids and the growth stages were explored (*).

(*) *Abbreviations:*
TCA = trichloracetic acid.
RNA = ribonucleic acid.
DNA = desoxyribonucleic acid.

Received for publication, October 15th, 1959.

Experimental

Material. Two *Neurospora crassa* strains were used (**). The wild type, which develops in minimal Fries medium with sucrose; and the 36703 mutant, which grows optimally in the same medium with 5×10^{-3} M to 10^{-2} M arginine. This mutant strain is genetically unable to synthesize arginine apparently because the enzyme implied in the synthesis of arginine-succinate is missing ([2]).

a) *Mycelia.* One or two drops of a conidia suspension were inoculated into 20 ml of minimal medium and were incubated at 25° C. In the mutant, suboptimal doses of arginine graded from 5×10^{-5} M to 7.5×10^{-4} M, ellicite a linear growth response.

In some experiments, the medium was supplemented with a mixture of 17 aminoacids including glycine, alanine, serine, threonine, leucine, isoleucine, valine, cysteine, methionine, phenylalanine, tryptophan, proline, aspartic and glutamic acids, lysine and histidine. The final concentration of each aminoacid was 25 mg per liter.

After 4 days, or at intervals during growth, mycelia from several flasks were harvested by smooth suction and washed with distilled water. The pads were squeezed on filter paper and placed over ice on Petri plates.

Dry weight was determined in an aliquot of the pad dried at 90° C for 24 hours. The residual fresh pad, weighing 30-250 mg, was finely minced and ground with Pyrex glass powder and 9 parts in volume of 0.002 M manganese sulphate. The homogenous milky fluid was centrifuged 5 minutes at 200 g.

A portion of the supernatant was used for arginine determination. The remainder was diluted 1:5 with the same Mn solution. This 1:50 (w/v) homogenate was used to determine total nitrogen, nitrogen precipitated by trichloroacetic acid and arginase activity.

It must be emphasized that these determinations were effected on the soluble fraction of mycelia extracted and homogenised as described. The figures obtained represent therefore the concentration or the content of components from this moiety and not the concentration or the content of the whole mycelial mass.

b) *Conidia.* Wild type conidia were obtained from cultures in solid minimal medium after 8-10 days at 25° C with intermitent lighting. Mutant 36703 cultures were enriched with 125 micrograms of arginine per ml of medium. Argininic acid ([3]) an structural analogue, was not a substitute for arginine.

Conidia were harvested and squeezed, then weighed and ground with glass powder and 50 volumes of 0.002 M manganese sulphate. Protein nitrogen and arginase activity were measured in this homogenous extract.

Methods. Total Nitrogen. 0.5 to 1.0 ml of mycelia homogenate was digested with sulphuric acid and nesslerized.

Protein Nitrogen. 0.5 to 2.0 ml of 1:50 homogenate was treated with 5 volumes of 10 per cent TCA. After standing several hours at 4-6° C the mixture was centrifuged and the sediment washed with the same TCA solution. The precipitate was carefully dragged to a Kjeldahl flask with distilled water, digested with sulphuric acid and nesslerized. This was called protein nitrogen.

Arginase activity. 1.25 ml of 0.05 M arginine chlorhydrate in glycine buffer 0.1 M pH 10.14 and 0.5 ml of 1:50 homogenate (equivalent to 10 mg of fresh *Neurospora* pad) were incubated 30 min at 37° C. The final pH was 9.5-9.6.

(**) Our thanks are due to Dr. N. H. Horowitz for kindly supplying us with *Neurospora* strains.

The urea formed was photometrically measured by the Archibald reaction (4). An arginase unit is defined as the amount of enzyme that liberates 1 micromole of urea per ml of incubation medium under these standard conditions. Specific activity is computed in units per mg of dry mycelia or more generally in units per microgram of protein nitrogen. Total activity is the enzyme activity of the whole mycelia mass contained in 20 ml of culture.

Arginine. The procedure of Cabello and Prajoux (5) was used. 0.5 to 2.0 ml of 1:10 or 1:20 homogenate was digested in sealed glass vials with the same volume of 6 M HCl at 100° C. The digest was adjusted to pH 6.5-7.0 with 6 M NaOH and then diluted to 5-10 ml.

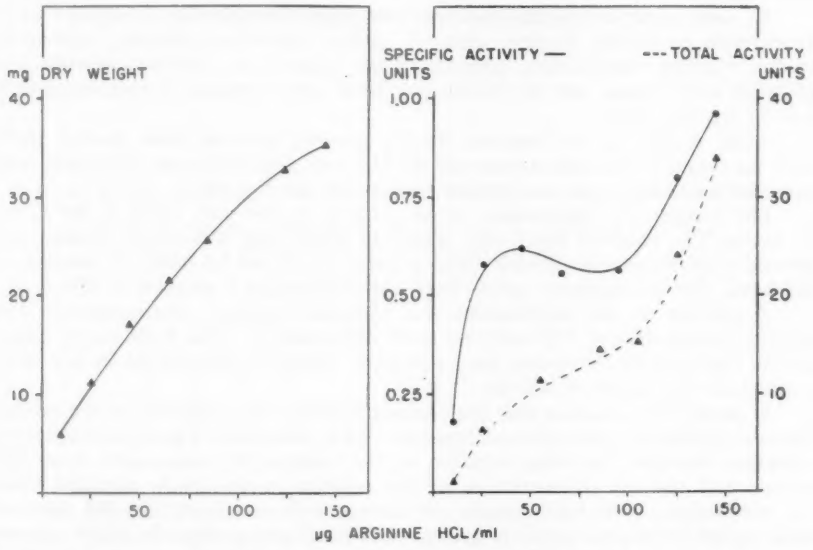


FIG. 1.—*Arginase activity of mutant mycelia after a 4-day culture in minimal medium supplied with increasing doses of arginine.* Specific activity: *Arginase units per mg of dry mycelium.* Total activity: *Arginase units per total dry mycelium formed in 20 ml of culture medium.*

2.0 ml of this neutral solution were incubated during 30 min at 37° C with 1 ml of a young rat liver 1:100 (w/v) homogenate, prepared in glycine buffer 0.1 M pH 10.12, and 0.04 ml of 0.2 M manganese sulphate. Under these conditions liver arginase was shown to liberate equimolecular amounts of urea from arginine. After deproteinization with 1.0 ml of 20 % metaphosphoric acid, urea was determined by the Archibald reaction.

The arginine content was computed by comparing the amount of urea evolved from 0.5 mg of arginine chlorhydrate in 2.0 ml water under the same conditions. Total arginine nitrogen is the amount determined in the mycelial mass formed in 20 ml of culture.

Exogenous Ammonia Nitrogen. It was determined by direct nesslerization of mycelia-free medium. 20 ml of minimal Fries medium contained 10.84 mg of ammonia nitrogen.

Results

1. *Arginase content of Conidia.*

As shown in Table I the specific arginase activity in wild conidia is of the same order of that usually found in fully developed wild mycelia (see Fig. 6). On the other hand, the mutant conidia have less than half of the arginase specific activity of wild conidia, although it increases rapidly with the germination and growth evoked by arginine.

2. *Arginase formation in cultures of N. crassa 36703.*

The effect of arginine on arginase formation was studied by growing the mutant in basal medium containing different concentrations of arginine and collecting the mycelia after a 4-day incubation.

Figure 1 shows the results obtained with 4 day-cultures. It can be seen that the increase of the arginase specific activity is not directly proportional to the growth, expressed as dry weight of mycelia.

The pattern of the specific activity curves were similar from one experiment to another, in spite of their different absolute values. On the other hand the total arginase activity increased with increasing the arginine concentration, but it was not strictly proportional to it.

The addition of a mixture of 17 aminoacids (see methods) caused an increase in the growth rate and total arginase content, but the shape of the specific activity curve was not affected.

It might be objected that in both sets of experiments the metabolic characteristics of the organisms were not equivalent because the growth stages depended on the amount of arginine supplied. So it seemed necessary to consider the pattern of arginase formation at different periods of the mutant growth and the influence exerted on it by the addition of aminoacids and arginine at various concentrations. To test these possibilities the following experiments were performed: a) action of aminoacids mixture on arginase formation when the mutant cultures were supplied with a fixed initial dose of arginine, b) effect of two different concentrations of these aminoacids on the enzyme synthesis at the same fixed arginine supply, c) effect of several arginine concentrations on arginase production, with and without addition of the aminoacid mixture.

a) The pattern of arginase formation during the mutant growth was determined by comparing the growth rates and the arginase contents of cultures developing in basal medium containing 25 μg/ml of arginine chlorhydrate, with and without the aminoacid mixture. Figure 2 shows the changes of arginase specific and total activities during development and the effect of aminoacids addition. It can be seen that the general shape of these curves was the same regardless the presence or the absence of exogenous aminoacids. A prominent feature was the rapid increase of the specific activity between 18 to 24 hours, period which corresponded to the end of the lag phase and the onset of the exponential phase. It reached a maximum value at 24 hours and declined steeply until 48 hours. Afterwards, it diminished gradually to a minimum at 7-8 days. The sole effect of the aminoacid addition was to depress the sudden rise of the specific activity attained during the lag exponential phase.

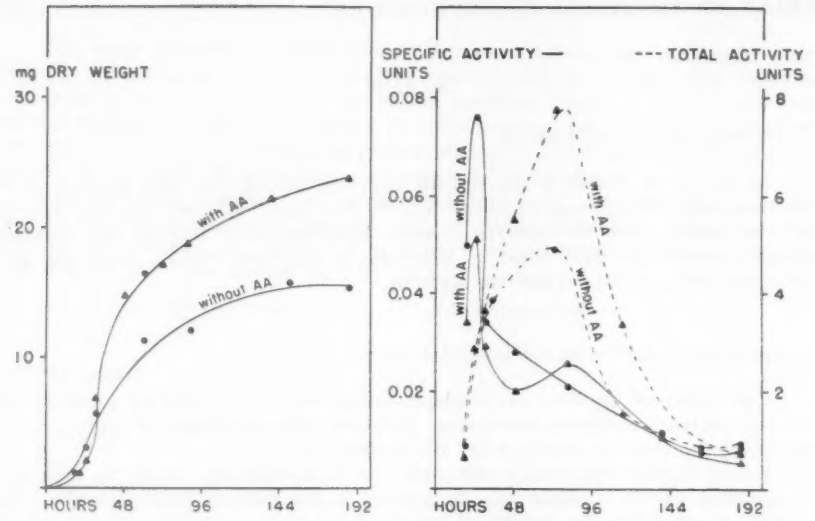


FIG. 2. — *Arginase production and its modification by aminoacids during mutant growth. Long term experiment, with 6-hours determinations between 18 and 48 hours. Minimal medium always contained 25 µg of arginine chlorhydrate per ml. In the experiment with aminoacids, the medium was supplied with a mixture of 17 aminoacids in the proportion of 25 µg per ml of each one.* Specific activity: *Arginase units por µg of mycelia protein nitrogen.* Total activity: *Arginase units por total protein nitrogen of mycelia formed in 20 ml of culture medium.*

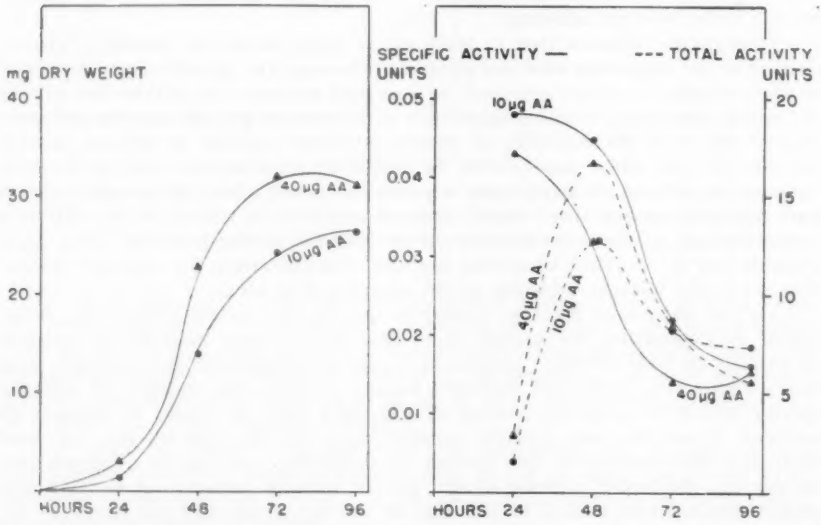


FIG. 3. — *Mutant growth and arginase production as influenced by the addition of two different doses of aminoacids to a culture medium of fixed arginine concentration (25 µg per ml). Aminoacids mixtures provided 10 and 40 µg of each aminoacid per ml of culture medium. Arginase specific and total activities as in Fig. 2.*

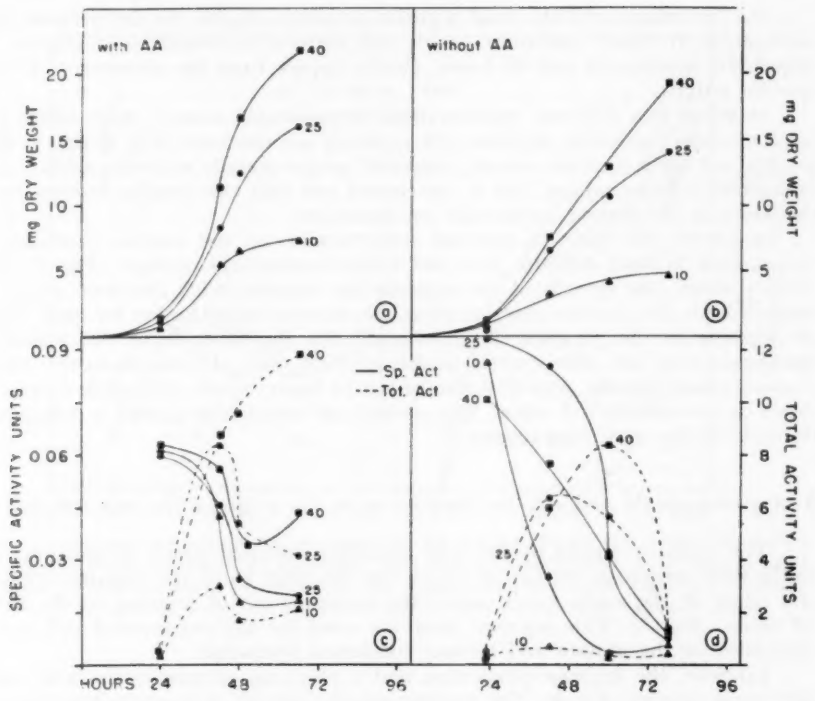


FIG. 4. — *Growth and arginase production in the mutant strain as a function of arginine dose and their modification by aminoacids. Arginine doses in culture medium: 10, 25 and 40 μg per ml.* Growth curves: *a) medium supplied with aminoacids, b) medium without aminoacids. Arginase specific and total activities as defined in Fig. 2, c) medium supplied with aminoacids, d) medium without aminoacids.*

TABLE I

Specific arginase activity of Neurospora conidia

Strain	Culture medium	Arginase units/μg protein N/30 minutes
36703	Minimal + Arginine (*)	0.016
Wild	Minimal	0.041

(*) 12.5 mg of Arginine hydrochloride per 100 ml of medium.

On the other hand the total arginase activity is higher in the presence of aminoacids. In both conditions —with and without aminoacids— it reaches a maximum between 48 and 98 hours, clearly appart from the maximum of the specific activity.

b) When two different concentrations of aminoacid mixture were added to basal medium containing arginine (25 μg/ml) it was observed (Fig. 3) that both, growth and total arginase content, increased proportionally with the amount of aminoacid mixture added; but it was found out that the specific activity was depressed at the highest aminoacids concentration.

c) Finally the effect of arginine concentration on the enzyme production was studied in basal medium with and without aminoacid mixture (Fig. 4). As already stated, the growth of the arginine-less mutant 36703 increased proportionally with the arginine concentration, the increase being higher for each dose of arginine in the presence of aminoacids. On the other hand the arginase specific activity was independent of the arginine doses. It was confirmed that a maximum of specific activity is attained at 24 hours, which afterwards dropped down to a stationary low value. The presence of aminoacids caused a consistent decrease of this maximum level.

3. *Arginine uptake and arginase production by the wild and the mutant strains.*

The arginine uptake by the wild organism increased nearly proportionally to the body weight on the earlier stages. On the other hand, the arginine uptake was larger in the mutant and caused the accumulation of arginine in the first 24 hours (Fig. 5). This arginine pool was used for the exponential cell multiplication of the mutant and became consumed afterwards.

Likewise, the arginase production had a dissimilar pattern in the wild and the mutant strains (Fig. 6). The arginase specific activity of the wild *Neurospora* was low in the first 24 hours but it increased rapidly from thereon. On the other hand, the arginase specific activity of the mutant was highest at 24 hours but it disminished slowly later on. The general shape of these curves was not modified by the addition of aminoacids. However, in the mutant the aminoacids supply tended to lower down the initial rise of the arginase specific activity.

The total arginase content of the whole mycelia mass increased as did the body weight in the wild strain, but it remained nearly stationary in the mutant strain.

Other metabolic characteristics of these *Neurospora* strains are assembled in Table II.

Discussion

The experiments reported here were mainly concerned with the synthesis of additional quantities of arginase in an arginine-less mutant of *Neurospora crassa*. The additional arginase was related with the concentration of arginine in the growth medium and with the different stages of the cultures.

The evidence presented can be summarized as follows: a) although the arginase specific activity in the mutant conidia amounts to 39 % of that of the wild type conidia, it increases more rapidily and surpasses that of the wild type during germination, b) the mycelial mass and the arginase content of 4-day

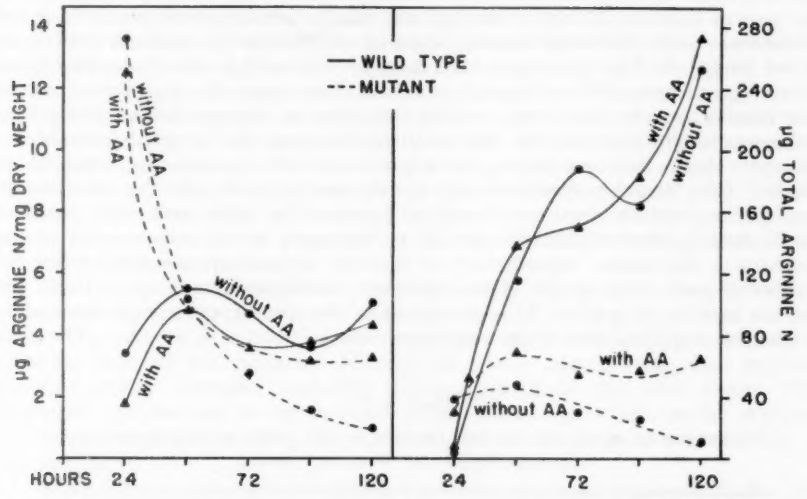


FIG. 5.—*Concentration and content of arginine in the mycelia of wild and mutant* Neurospora *strains, and their modification by aminoacids. The arginine concentration is expressed as μg of arginine nitrogen per mg of dry mycelium. The arginine content or total arginine is expressed as μg of arginine nitrogen in the whole mycelia formed in 20 ml of culture medium.*

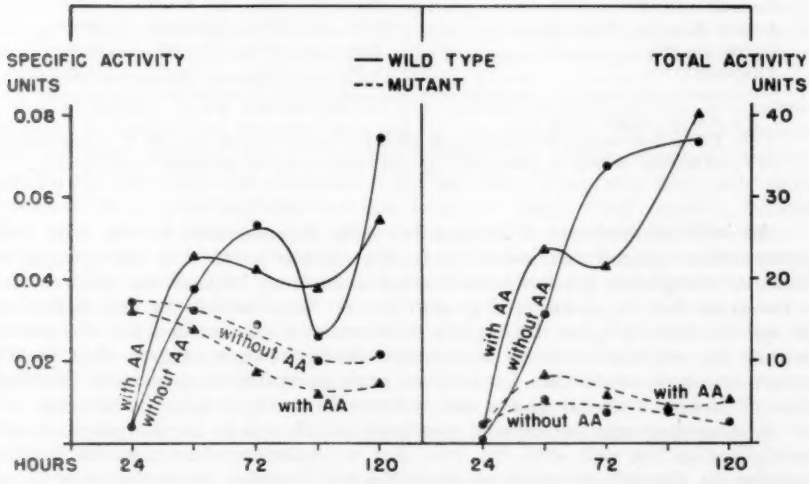


FIG. 6.—*Arginase synthesis in wild and mutant* Neurospora *strain and the effect therein of aminoacids added to the culture medium. Arginase specific and total activities as in Fig. 2.*

cultures of the mutant, increase proportionally to the concentration of arginine in the growth medium. However, the arginase specific activity grows better than the tissue mass when the initial concentration of arginine in the medium is between 5 and 20 μg/ml. This particular behaviour is attributed to the limited synthesis of average proteins at low arginine concentrations, thus the arginase would be less diluted out by the other cellular proteins, c) independently of arginine concentration in the medium, the total arginase and the mycelial mass of the mutant cultures increase during the exponential and the negative acceleration phases. They decrease slowly during the stationary phase. On the other hand, the specific activity increases abruptly between the 18th and 24th hour of incubation (period which corresponds to the onset of the exponential phase) reaching a maximum. Subsequently it declines throughout the rest of the exponential and other stages. The maximum corresponds to 4 to 5 times the specific activity of conidia. This sharp rise in the specific activity persists longer in cultures supplied with higher arginine doses.

TABLE II

Uptake and utilization of ammonia nitrogen by two strains of Neurospora crassa
(Average figures for 96 hours cultures)

	Wild strain		36703 mutant strain	
	Without AA (*)	With AA	Without AA	With AA
Dry mycelia ..... mg	49.10	62.00	13.40	19.60
Ammonia N consumed ..... mg	3.52	3.89	0.85	1.30
Total soluble N ..... mg	1.03	1.19	0.34	0.61
Protein soluble N	0.64	0.85	0.15	0.35
Protein N as % of total N	62.00	71.00	43.00	59.00
Utilization (**)	5.50	4.50	5.80	3.60
Efficiency (***)	0.18	0.22	0.17	0.28

(*) AA = Amino acids.
(**) Utilization = mg of ammonia N invested when 1 mg of protein N is synthesized.
(***) Efficiency = mg of protein N builded when 1 mg of ammonia N is uptaken.

An induced synthesis of arginase has been demonstrated for the wild type grown under optimal conditions ([1]). In the mutant strain it is not possible to dissociate completely growth from enzyme induction, because the inducer acts at the same time as an essential growth factor. Nevertheless, the dependence of the specific activity upon the arginine concentration in the medium which may increase the arginase over the constitutive level, strongly suggests that in the mutant strain, a mechanism for induced arginase synthesis also exists. Whether this mechanism is similar to the one in the wild strain, remains to be seen.

The steeper rate of arginase synthesis which occurs at the onset of cell multiplication fits well with the idea that arginase serves an essential linking function in the cell metabolism. Probably the function concerned here is to supply from arginine the precursors of aminoacids and pyrimidine bases through the ornithine cycle. This interpretation is parallel to the observations that a

maximum of biochemical activities such as CO_2 and NH_3 production ([6]) and RNA synthesis ([7]) is attained during the lag phase.

The mutant strain, but not the wild type, accomplished a bulky accumulation of arginine, followed by its utilization, at the exponential period. There was a concomitant rise of the arginase specific activity. The subsequent rapid decay of this enzyme activity can be explained by the internal breakdown of arginine and the reduction of its external concentration. The removal of the products of intracellular hydrolysis shifts the reaction equilibrium to a larger arginine destruction, reducing in this way the internal concentration of the inducer substrate. An alternative explanation is that the actively growing cells have accumulated analogues of arginine (or of the effective inducer) which block the inductive power.

On the opposite, the wild strain synthesized arginine and the complementary aminoacids from mineral nitrogen and used them to build proteins and nucleic acids. The general expansion of these synthetic mechanisms did not require a previous internal accumulation of arginine. As a consequence, the arginase specific activity increased smoothly during growth of the wild strain. The destroyed arginine can be continuously rebuilded in this strain, so the arginase activity stands higher during the stationary phase, keeping its normal rôle in cell anabolism.

Some facts about enzyme induced synthesis have been disclosed: 1) the cell pool of free aminoacids is the source of new formed protein ([8, 9, 10, 11]); 2) a pool of simple nucleotide seems to be needed ([12]); 3) RNA, but not DNA is required ([13]); 4) there are specific cell acceptors where the inducer is fixed ([14, 15]).

The enzyme synthesis utilizes the free aminoacid pool and the first stable intermediate must be seen as complex as a protein ([8]). The experiments of Rotman and Spiegelman ([16]) and those of Hogness, Cohn and Monod ([17]) indicate that the split products of protein catabolism are not used. Protein synthesis appears to be an irreversible process. Some restriction to this concept emerges from Mandelstam's work ([18, 19]) demonstrating that proteins are stable in rapid growing cells of Escherichia coli, but not in non-growing cells which exhibit a detectable protein turnover.

The supply of 17 aminoacids to the culture medium of the *Neurospora* mutant, in suboptimal growth, increases the mycelia mass, the soluble proteins and the total arginase, but it partially inhibits the sudden burst of arginase specific activity when the exponential period starts. It appears that aminoacids discharge the arginine-arginase reaction from its "single road" quality, because they open new accesses for the synthesis of essential metabolites. This effect is consistent with the here admitted physiological rôle of arginase in the mutant strain. When aminoacids are provided, the removal of the products of arginine hydrolysis become delayed and the arginine incorporation on average proteins enhanced. Then the amount of inducer gets down and the arginase specific activity does not rise to a so high lag-exponential level as in the mutant cultures deprived of aminoacids. However, the occurrence of a set of aminoacids enhances the synthesis of new arginase molecules and causes, in the mutant, a later sustained high level of specific activity, which is directly proportional to the amount of aminoacids added.

The synthesis of nucleotide bases requires some aminoacids, as glycine, aspartic and glutamic acids. The mutant, when only supplied with arginine, elaborates the nucleotide precursors from the hydrolytic products of arginase

action. When the aminoacid mixture is added, the nucleotide synthesis becomes not absolutely dependent from arginine metabolites, then the general protein synthesis is improved and competes with arginase synthesis during the exponential phase.

When the mixture of aminoacids is given to the wild strain it causes a moderate increase of mycelial mass but produces no definite modification of the total and specific activity of arginase.

Even though a demonstration of an induced synthesis of arginase in classical terms is practically impossible in the arginine-less mutant of *Neurospora crassa*, we could infer from the preceding data that the additional fraction of enzyme formed is adaptive and that the substrate acts as an inducer.

Our findings suggest a new insight into the mechanism of induced enzyme synthesis.

The intracellular activity of a constitutive enzyme is relatively constant because the inducer substrate to which it combines is a normal metabolite in a steady concentration. It has been shown possible, however, to increase largely the amount of a constitutive enzyme in animal tissues, by the administration of an excess of a normal metabolite ([20, 21]), a reaction essentially similar to enzyme adaptation in microorganisms. The enzyme overproduction appears as a consequence of a greater rate of the release turnover of the enzyme at the surface of its nucleoprotein template, with the subsequent accelerated apposition of new enzyme molecules on this template by lining and binding the whole aminoacids.

The adaptive enzymes are always cell constituents existing in so small amount that its demonstration can require refined techniques. This tracer concentration, indicating the presence of a small template, is several times increased by the action of an inducer (substrate or non-substrate) that forms a complex with the enzyme and increases the enzyme delivery rate from the template. By this mechanism, the synthesis of new enzyme molecules is started and hastened.

The fact that the neoformed enzyme dissapears when the inducer is removed demonstrates that the template size does not change during induction but only its functional turnover. However, it is not excluded that in uncommon cases, the magnitude of the template is also expanded and the enzyme synthesis may proceed longer time at a high rate, as occurs in the Pollock phenomenon ([22]).

On this ground, we think that the accessory amount of arginase formed by the *Neurospora* mutant as a function of the increasing arginine concentration in the medium and its acute rise when cell multiplication begins to accelerate, implicates a reaction which cannot be distinguished from enzyme adaptation.

Summary

1. Arginase is a constitutive enzyme of conidia and mycelia in an arginine-less mutant of *Neurospora crassa*, whose concentration is adaptively increased by an inducer action of the substrate. In our experimental conditions, it has not been possible to dissociate enzyme synthesis from growth, because the substrate is also an essential growth factor.

2. The arginase specific activity of this *Neurospora* mutant increases to a maximum value at the onset of the exponential growth phase, when arginine is also accumulated into mycelia. These peculiar reactions are not shown by *Neurospora* wild strain.

3. The variations of arginase specific activity during the growth phases and the effect of the supply of a mixture of 17 aminoacids on these changes, demonstrate an important linking role of arginase on the mutant biochemical synthesis. Splitting of arginine sets in motion production of metabolites required for building of proteins and nucleotides, from which the chemical machinery of the cell is organized.

4. On a theoretical approach, we consider as an anzyme inducer any compound that speeds the delivery turnover of the enzyme from its ribonucleoprotein matrix, through a complex-forming mechanism; we qualify as enzyme adaptation the process in which enzyme synthesis is promoted by acceleration of this turnover.

References

(1) Srb, A. M., Horowitz, N. H.: *J. biol. Chem.*, 1944, *154*, 129.
(2) Fincham, J. R. S., Boylen, J. B.: *J. gen. Microbiol.*, 1957, *16*, 438.
(3) Felix, K., Schneider, L.: *Zeit. physiol. Chem.*, 1938, *255*, 132.
(4) Archibald, R. M.: *J. biol. Chem.*, 1945, *157*, 507.
(5) Cabello, J., Prajoux, V.: *Acta physiol. lat. amer.*, 1956, *6*, 38.
(6) Winslow, C. E. A., Walker, H. H.: *Bact. Revs.*, 1939, *3*, 147.
(7) Morse, M. L., Carter, C. E.: *J. Bacteriol.*, 1949, *58*, 317.
(8) Halvorson, H., Spiegelman, S.: *J. Bacteriol.*, 1952, *64*, 207.
(9) Halvorson, H., Spiegelman, S.: *J. Bacteriol.*, 1953, *65*, 796.
(10) Halvorson, H., Fry, W., Schwemmin, D.: *J. gen. Physiol.*, 1955, *38*, 549.
(11) Stanier, R. Y.: *The Synthesis of Enzymes in Outlines of Enzyme Chemistry*; J. Wiley and Sons, N. York, 1955.
(12) Reiner, J. M., Goodman, F.:*Arch. Biochem. Biophys.*, 1955, *57*, 475.
(13) Halvorson, H., Jackson, L.: *J. gen. Microbiol.*, 1956, *14*, 26.
(14) Cohen, G. N., Rickenberg, H.: *C. R. Acad. Sci.* (Paris), 1955, *240*, 466.
(15) Monod, J.: *Exposés Ann. Biochim. Méd.*, 1955, *17*, 195.
(16) Rotman, B., Spiegelman, S.: *J. Bacteriol.*, 1954, *68*, 419.
(17) Hogness, D., Cohn, M., Monod, J.: *Biochim. Biophys. Acta.*, 1955, *16*, 99.
(18) Mandelstam, J.: *Biochem. J.*, 1958, *69*, 103.
(19) Mandelstam, J.: *Biochem. J.*, 1958, *69*, 110.
(20) Knox, W. E., Auerbach, V. H.: *J. biol. Chem.*, 1955, *214*, 307.
(21) Lin, E. C. C., Knox, W. E.: *Biochim. Biophys. Acta.*, 1957, *26*, 85.
(22) Pollock, M. R.: *Brit. J. exp. Path.*, 1950, *31*, 739.

LAS CONTRACCIONES ISOTÓNICAS DE LOS MÚSCULOS ESTRIADOS

RAFAEL RUBIO G.

(Sección de Investigación de Medicina Física, Hospital Infantil de México, México)

TANTO el músculo como la fibra muscular estriada son sistemas elásticos heterogéneos. Incluyen elementos elásticos pasivos en serie y en paralelo con los elementos contráctiles.

Varios autores ([1, 2, 5]) han hecho notar que la existencia de los elementos pasivos en serie hace imposible que el sistema contráctil se active isométricamente.

El propósito del presente trabajo fué analizar el papel de los elementos pasivos en paralelo, usando fibras musculares que no eran activadas al aplicar pulsos eléctricos al nervio motor. Que los elementos pasivos en paralelo impiden el desarrollo de contracciones isotónicas ha sido mencionado por Hill ([3]).

MÉTODO

Se emplearon ratas, anestesiadas con nembutal (50 mg/kg) por vía intraperitoneal. El músculo elegido fué el gastrocnemio, liberado del sóleo. Se machacó el nervio poplíteo y se le colocó, en el cabo distal, un par de electrodos para estimulación. La pata se fijó en los puntos apropiados y se unió, por medio de un hilo, el tendón de Aquiles a una palanca de tipo isotónico que inscribía sobre un quimógrafo.

Los estímulos empleados fueron pulsos rectangulares de intensidad máxima y de frecuencia entre 50 y 70 por segundo. Los períodos de estimulación fueron lo suficientemente prolongados para obtener el máximo de las respuestas.

En unos experimentos, se colocaron dos pares de electrodos de estimulación al poplíteo y se hizo una sección parcial del nervio entre ellos, de tal manera que a través del par inferior se activaban todas las fibras musculares y con el par superior, sólo una fracción.

Recibido para publicación, Agosto 15, 1959.

En otros experimentos, se registraron independientemente las respuestas de cada uno de los gemelos; en estos casos, siempre se activaron todas las fibras musculares.

Resultados

I. *Diagramas de tensión-longitud, obtenidos independientemente de cada uno de los gemelos.* La figura 1 muestra los diagramas de tensión-longitud (T-L) correspondientes a ambos gemelos. En estos diagramas, al igual que en las siguientes figuras, las ordenadas representan la longitud del músculo y las abscisas la carga a la cual se encuentra sometido. La curva inferior es el diagrama de T-L de reposo y la curva superior, el correspondiente al músculo estimulado.

Las cruces de la figura 1 corresponden al diagrama de T-L obtenido para la fracción muscular más pequeña y los círculos, al obtenido para la fracción mayor. Para lograr esta superposición aceptable entre los diagramas de T-L de ambas fracciones musculares, se emplearon dos escalas en el eje de las cargas, cuya razón es de 4.5; la escala superior de la figura 1, corresponde a las cruces

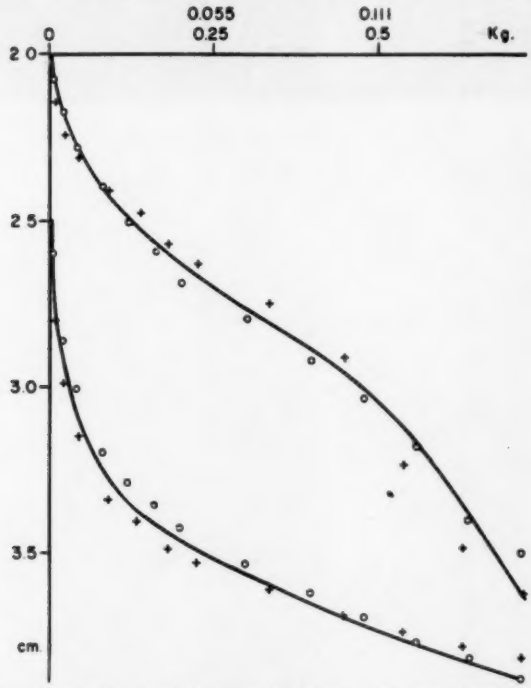


Fig. 1.—*Diagramas de tensión-longitud, correspondientes a los gemelos de un mismo gastrocnemio; estos diagramas fueron tomados simultánea e independientemente. Las cruces representan los diagramas de T-L de la fracción muscular más pequeña (0.285 g), los círculos correspondientes a la fracción más grande (1.3 g). La escala superior de las abscisas, corresponde a las cruces y la inferior, a los círculos. En todas las figuras de este trabajo, la curva superior representa el diagrama de T-L del músculo contraído y la curva inferior, el músculo en reposo.*

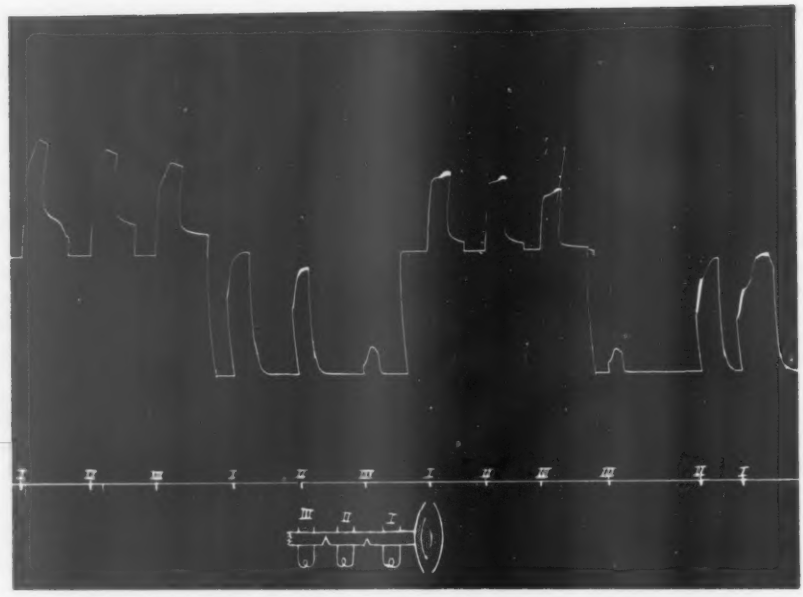


FIG. 2. — *Registro quimiográfico de los acortamientos de un gastrocnemio al aplicar estímulos en 3 pares de electrodos, colocados en el nervio motor, I, II y III, según el diagrama inferior de la figura. En el espacio existente entre cada par se hizo una sección parcial del nervio. El número romano abajo de cada respuesta indica el par a través del cual se originó ésta. El primer y tercer trío de respuestas se obtuvieron con 20 gramos; el segundo y cuarto trío, con 500 gramos.*

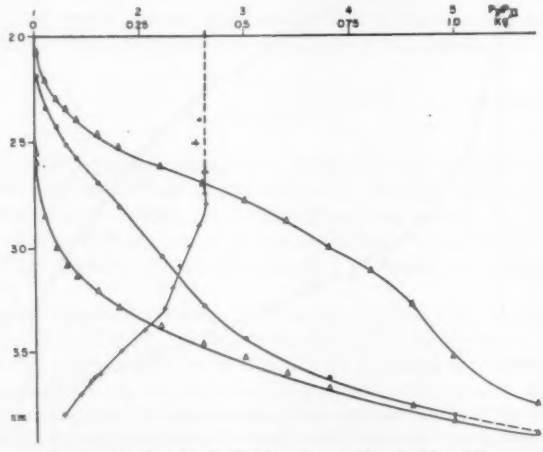


FIG. 3. — *Diagramas de tensión-longitud. Triángulos: estimulación del 100 por ciento de las fibras. Puntos: activación de sólo una fracción de ellas. La curva de las cruces, corresponde a la razón que hay entre las cargas de las curvas de puntos y la superior de triángulos. La escala superior de las abscisas, se refiere a la curva de cruces.*

y la inferior a los círculos. La razón de 4.5 es aproximadamente igual a la que había entre las masas de ambas fracciones musculares. Estos resultados, están de acuerdo con la idea de que se puede reducir la forma geométrica, de ambos gemelos, a dos cilindros de igual altura y diferente área de sección, puesto que, al estar constituídos por la misma substancia, las áreas de sección están en razón directa a las masas musculares.

II. *Efecto de la carga y del número de elementos pasivos en paralelo sobre la magnitud de la respuesta isotónica.* Se colocaron tres pares de electrodos en el nervio motor como indica el diagrama en la parte inferior de la figura 2. En la separación existente entre cada par se hizo una sección parcial del nervio, siendo mayor la sección entre los pares II y III que entre los pares I y II. Al estimular en II y en III las fibras musculares que no eran activadas actuaban como elementos pasivos en paralelo, siendo mayor este número de elementos al estimular en III. El registro fué hecho tomando toda la masa muscular.

El primer grupo de respuestas de la figura 2 fué obtenido al estimular separadamente a través de cada uno de los pares y estando el músculo sometido a una carga de 20 gramos. El segundo grupo, fué obtenido estando el músculo sometido a una carga de 500 gramos; el tercer y cuarto grupos son una repetición del primero y segundo.

Al comparar las respuestas obtenidas estimulando en II y III, con las obtenidas estimulando en I, es claro que la discrepancia en la magnitud de las respuestas se acentúa al aumentar la carga. Esta discrepancia es más acentuada mientras mayor es el número de elementos pasivos en paralelo, como lo indican las respuestas obtenidas al estimular en III.

III. *Diagramas de T-L al introducir elementos pasivos en paralelo.* Los datos de estos diagramas fueron obtenidos colocando dos pares de electrodos al poplíteo y haciendo entre ellos una sección parcial del nervio. Los resultados se ilustran en la figura 3. La curva inferior corresponde al diagrama de T-L de reposo, la curva media (puntos), al obtenido al estimular a través de los electrodos distales, y la curva superior de triángulos, al obtenido al aplicar los estímulos al par proximal y activar todas las fibras musculares.

La razón P_I/P_{II} que existe para una longitud dada entre las cargas que tienen a esa longitud los diagramas de T-L de músculo contraído (curvas de puntos y triángulos) graficada contra la longitud, está representada por la curva de cruces. La escala superior de las abscisas corresponde a la razón P_I/P_{II}. Este cociente no fué constante en ninguno de los experimentos.

En el experimento ilustrado en la figura 3 el número de fibras musculares que actuaban como sistemas elásticos pasivos en paralelo corresponde al 64 por ciento de la masa muscular; los elementos activos eran el 36 por ciento. En la discusión se indicará cómo se determinan estos porcentajes.

Discusión

Llamemos P_{II} (L_o) a la carga que soporta el músculo cuando al acortarse al máximo adquiere longitudes próximas a 2.5 centímetros. Esta es la longitud que se asignó a los músculos estudiados en reposo y con carga cero. Si se estimula sólo un grupo de fibras musculares, las no estimuladas se encuentran en equilibrio a esta longitud con carga cero y, por consiguiente, toda la carga P_{II} (L_o) es soportada por las fibras que han sido estimuladas. Al estimular todas las fibras, la carga P_I (L_o) necesaria para que el músculo al acortarse al máximo adquie-

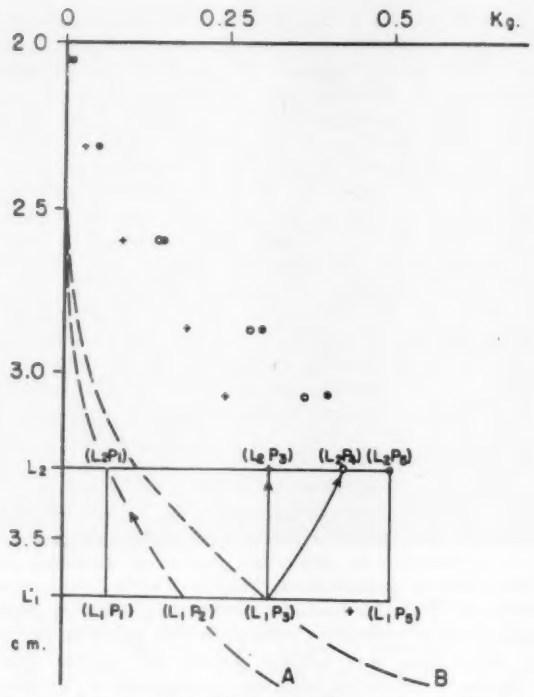


FIG. 4.—*Para la explicación de esta figura véase la discusión.*

ra la longitud de 2.5 centímetros es mayor que P_{II} (L_o) (fig. 3). En la sección I se mostró que para una longitud dada, la razón de las cargas que soportan dos masas musculares a esa longitud ya sea contraídos o en reposo es igual a la razón directa de las masas musculares (fig. 1). Por lo tanto, la razón de las cargas P_{II} (L_o) y P_I (L_o) nos dice qué porcentaje de la masa muscular total representa el grupo de fibras activadas al aplicar estímulos a través del par de electrodos colocados después de la sección parcial del nervio; la diferencia de este porcentaje con cien, representa el correspondiente a los elementos que actuaban como pasivos en paralelo.

Los datos de la figura 4 corresponden a un experimento en el que el 37 por ciento de las fibras musculares actuaban como elementos pasivos en paralelo y el 63 por ciento como elementos activos. Considerando los datos presentados en la sección I podemos decir que a cualquier longitud en el diagrama de T-L de reposo del músculo, el 37 por ciento de la carga era sostenido por las fibras que no se contraen y el 63 por ciento por las que se activan. Las curvas A y B de la figura 4 representan los diagramas de T-L de reposo del 37 y 63 por ciento de las fibras del músculo. Los puntos de la figura 4 representan el diagrama de T-L del músculo contraído obtenido al aplicar estímulos a través del par de electrodos colocado después de la sección parcial del nervio.

Supongamos que se cuelga al músculo una carga igual a P_5 que lo estirá

hasta L_1; a esta longitud los elementos activos quedan en equilibrio con la carga P_3 y los pasivos con P_2, ambas cargas son el 63 y 37 por ciento de P_5, respectivamente. Al estimular, las fibras activas y las no activas se acortan hasta L_2; a esta longitud, los elementos pasivos están en equilibrio con la carga P_1 y los activos con la carga $P_5 - P_1 = P_4$, puesto que la carga en los extremos del músculo debe permanecer constante. Esto nos muestra que los elementos activos no se acortan siguiendo la vertical (L_1, P_3) (L_2, P_3) sino siguiendo la curva (L_1, P_3) (L_2, P_4), o sea, al acortarse no lo hacen isotónicamente sino con un aumento continuo de carga.

Los puntos marcados por cruces en la figura 4 representarían la curva de las cúspides si al estimular el grupo activo unido en paralelo con el grupo pasivo, se contrajeran en forma isotónica, pero considerando que hay un incremento de carga para los elementos activos, este incremento desplaza las cúspides hacia los puntos marcados por círculos, que se encuentran en la horizontal correspondiente a cada cruz. Todo el razonamiento anterior explica por qué en la figura 2 la discrepancia en la magnitud de las respuestas obtenidas al estimular a través de los pares II y III comparado con los obtenidos estimulando en I, se acentúan al aumentar la carga y explica, además, por qué esta discrepancia es más acentuada mientras mayor es el número de elementos pasivos en paralelo. Todo esto es debido a que las formas de las curvas son tales que un pequeño acortamiento de los elementos activos a cargas grandes determina para ellos un incremento grande de carga y este incremento es mayor al aumentar el número de elementos pasivos.

Se dijo que al estimular el grupo activo, estando éste unido en paralelo con el grupo pasivo, se obtiene la curva representada por puntos en la figura 3. Estos puntos no representan el diagrama de T-L del grupo activo puesto que están graficadas las longitudes L_2 contra las cargas P_5; el diagrama que representa al grupo activo estimulado es el que grafica L_2 contra P_4, o sea, el correspondiente a los círculos (fig. 4). Esto explica por qué la razón P_I/P_{II} de la figura 3 no es una constante; P_{II} es en este caso igual a P_5, si se graficase P_I/P_4 se encontraría que es una constante. Esta es una inferencia sacada al considerar lo comunicado en la sección I.

Ya se dijo que la curva obtenida al estimular al grupo activo estando unido en paralelo al grupo pasivo, no representa el diagrama de T-L del primero. Contamos con dos métodos para la determinación del diagrama de T-L del grupo activo.

1º Si se conoce el porcentaje de elementos activos y el diagrama de T-L correspondiente a las cúspides del total de los elementos musculares, como justifica la sección I, basta graficar las longitudes de este diagrama, no contra las correspondientes cargas totales, sino contra el porcentaje de las cargas igual al porcentaje de elementos activos.

2º La curva obtenida al estimular el grupo activo estando unido en paralelo al grupo pasivo, no representa el diagrama de T-L del primero porque, como ya se dijo al analizar la figura 4, esta curva corresponde a (L_2, P_5), mientras que la correspondiente al diagrama de T-L es la de (L_2, P_4). El procedimiento para calcular P_4 fué el siguiente: conociendo los diagramas de T-L de reposo del grupo pasivo y del activo (curvas A y B) y los acortamientos máximos (L_2-L_1) que sufre el músculo al ser estimulado a las diferentes longitudes iniciales (L_1), se pueden determinar los incrementos de carga sobre P_3 que sufren los elementos activos al acortarse; estos incrementos son iguales a los decrementos

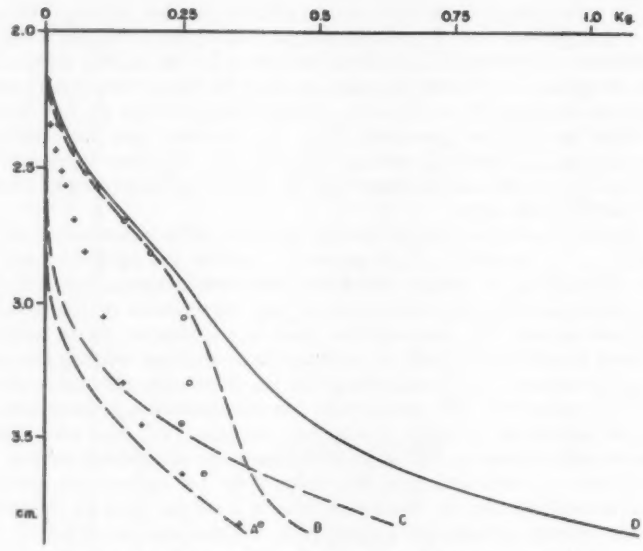


FIG. 5. — *Para la explicación de esta figura véase la discusión.*

que ocurren en los pasivos a partir de P_2, decrementos representados en la figura 4 por P_2-P_1. Así la carga P_4 queda determinada al sumar P_2-P_1 a P_3.

Las curvas de la figura 5 fueron construídas con los datos de la figura 3. Las curvas A y C describen los diagramas de T-L de reposo, correspondientes a los elementos activos y pasivos respectivamente; como ya se dijo, los elementos activos representaban el 37 por ciento de las fibras musculares y el 63 por ciento restante correspondía a los pasivos. La curva B corresponde al diagrama de T-L del 37 por ciento de los elementos activos, calculada aplicando el primer método arriba mencionado. La curva D es la experimental obtenida al estimular los elementos activos estando unidos en paralelo con los pasivos. Los círculos corresponden a las relaciones (L_2, P_4) calculados al aplicar el segundo método. Los puntos marcados con cruces tienen la misma representación que en la figura 4. La curva B, como la correspondiente a (L_2, P_4), representa el diagrama de T-L de los elementos activos y, por lo tanto, deberíamos esperar una superposición de ambos. Los círculos se superponen en la curva B para las cargas pequeñas y no para las grandes. La desviación con las cargas grandes es explicable al tomar en cuenta la histéresis de las fibras que no se contraen; considerando este factor, los círculos con cargas grandes deberían correrse a la derecha, puesto que el incremento de carga sería mayor.

Apoya esta idea de la histéresis el hecho de que la desviación de los círculos a la curva teórica ocurrió siempre a cargas grandes.

Resumen

La forma como está estructurado el músculo, hace imposible obtener contracciones isotónicas o isométricas puras.

Se empleó el gastrocnemio de la rata. En unos experimentos se registró independientemente de cada uno de los gemelos, aplicando estímulos máximos al poplíteo con frecuencias de 50 a 70 por segundo. Los diagramas de tensión-longitud (T-L) de las dos fracciones, se superponen al ajustar las escalas de los ejes de las tensiones, de tal modo que guarden la misma razón que los pesos musculares.

En otros experimentos, se colocaron dos pares de electrodos de estimulación en el poplíteo y se hizo una sección parcial del nervio entre ellos. El par inferior activaba todas las fibras motoras, el superior, sólo una fracción. Los diagramas de T-L no se pueden ajustar como antes. Conociendo el diagrama T-L de reposo de las fibras que no se contraen, se puede corregir el correspondiente a la fracción de fibras activas y obtener el diagrama real de éstas.

Estos resultados, indican que al acortarse un músculo cambia la distribución de la carga entre los elementos activos y los elásticos pasivos en paralelo. Como consecuencia, la contracción no es isotónica.

Summary

A study was made of the isotonic contraction of rat gastrocnemius muscles.

It was found that the structural organization of the muscle made it impossible to obtain strictly isotonic contractions.

In some experiments contractions were recorded independently from the medial and from lateral gastrocnemius. Stimuli were applied to the popliteal nerve at a frequency of 50-70/sec.

The tension-length (T-L) diagrams of the medial and lateral muscle can be superimposed by adjusting the abscissas so that the tension scales bear the same relation as that of the muscles weights (fig. 1).

In other experiments a partial section of the popliteal nerve was made. A pair of stimulatory electrodes were placed at each side of the section, so that of the central pair stimulated only a fraction of the motor fibers to the muscle, while the more distal one activated all of them.

The two (T-L) diagrams obtained in these conditions can not be adjusted as before. Nevertheless in the case that the muscle was activated through the central electrodes, the resting T-L diagram of the unstimulated fibers can be determined and a correction may then be made to the experimental T-L diagram to obtain that of the activated fibers (fig. 5).

These results show that, at shortening there is a change of the distribution of loads between the active and the passive parallel elements of muscle and so the contraction can not be isotonic (fig. 4).

Bibliografía

(1) Hill, A. V.: *Proc. roy. Soc. B.*, 1951, *138*, 325.
(2) Hill, A. V.: *Proc. roy. Soc. B.*, 1951, *138*, 339.
(3) Hill, A. V.: *Proc. roy. Soc. B.*, 1951, *138*, 329.
(4) Jewell, B. R., y Wilkie, D. R.: *J. Physiol.* (Lond.) 1958, *143*, 515.

LA DISTRIBUCIÓN DE LA TENSIÓN EN EL MÚSCULO DURANTE EL REPOSO Y LA CONTRACCIÓN

RAFAEL RUBIO G.

(Sección de Investigación de Medicina Física, Hospital Infantil de México, México)

RUBIO [9] analizó el papel que desempeñan en la contracción los tejidos elásticos pasivos en paralelo del músculo y sugirió que su existencia no hace posibles las contracciones isotónicas.

En un trabajo anterior, Rosenblueth, Alanís y Rubio [8] discutieron el diagrama de tensión-longitud del músculo contraído isométricamente, que han descrito Hill [5] y otros [10, 11]. En ese diagrama, al aumentar la longitud, la tensión del músculo contraído aumenta hasta alcanzar un máximo, luego cae para alcanzar un mínimo y empezar otra vez a crecer; con alargamientos grandes tiende a juntarse con el diagrama de tensión-longitud de reposo. En nuestros experimentos no encontramos diagramas con estas características y pensamos que, si son aceptados, habría que atribuirle a los músculos propiedades elásticas hasta ahora no descritas para ningún cuerpo. Interpretamos sus resultados como debidos a que al ir estirando progresivamente al músculo, se provocaron cambios no reversibles.

Por otra parte, se ha supuesto que la tensión del componente activo del músculo es igual a cero durante el reposo [1, 6, 7].

El presente trabajo fué planeado para intentar resolver estos problemas.

MÉTODO

Se emplearon ratas, anestesiadas con nembutal (50 mg/kg) por vía intraperitoneal. Se seccionó el nervio ciático al nivel del trocanter. Se disecó el poplíteo y se le pusieron en el cabo distal un par de electrodos para estimulación. El músculo empleado fué el gastrocnemio, liberado del soleo. Después de fijar la pata en los puntos apropiados se unió por medio de un hilo el tendón de Aquiles, que se había previamente desinsertado del hueso, a una palanca de tipo isotónico que inscribía sobre un quimógrafo. La duración de los estímulos fué de 0.5 a 1.0 milisegundos, y la intensidad dos veces la máxima, las frecuencias de estimulación fueron tetanizantes y su valor se indicará en cada caso.

RESULTADOS

A. *La irreversibilidad de los diagramas de tensión-longitud con cargas grandes.* La figura 1 muestra unos diagramas de tensión-longitud llevados hasta cargas donde se aproxima el diagrama de tensión-longitud del músculo contraído con

Recibido para publicación, Agosto 15, 1959.

el del músculo en reposo. Las abscisas representan la carga a la cual estaba sometido el músculo y las ordenadas los incrementos de longitud a partir de un cero arbitrario. Las dos curvas inferiores representan los diagramas del músculo en reposo; las cruces corresponden a los valores obtenidos al aumentar las cargas, como indica la flecha en la curva; los círculos representan al diagrama obtenido al disminuir la carga. Las dos curvas superiores muestran los diagramas correspondientes del músculo contraído.

El primer punto de esta figura corresponde a una carga de 700 g. El número de puntos, tanto de aumentos como de disminuciones, es pequeño. Esto se hizo con el propósito de evitar que el músculo se fatigase. Se tomó la precaución adicional de dar al músculo sin ninguna carga un reposo de 10 minutos entre cada período de estimulación. En lo referente a los diagramas de las cúspides, los experimentos mostraron en general que mientras mayor la carga máxima aplicada en los aumentos, más grande la discrepancia con el diagrama de las disminuciones. Al volver a aumentar las cargas se obtenían valores iguales a los de la curva de las disminuciones, o sea que el músculo quedaba en un nuevo equilibrio. Esto implica que no es por fatiga por lo que se van juntando los diagramas de tensión-longitud, ni es por fatiga por lo que el diagrama de las disminuciones queda por abajo del de los aumentos, porque de ser la fatiga la responsable, al volver a aumentar la carga el tercer diagrama quedaría todavía más abajo.

B. *La influencia de la longitud en la irreversibilidad de los diagramas de tensión-longitud.* La primera respuesta de la figura 2 fué obtenida estimulando a 60 por segundo, estando sometido el músculo a una carga de 100 g. Después

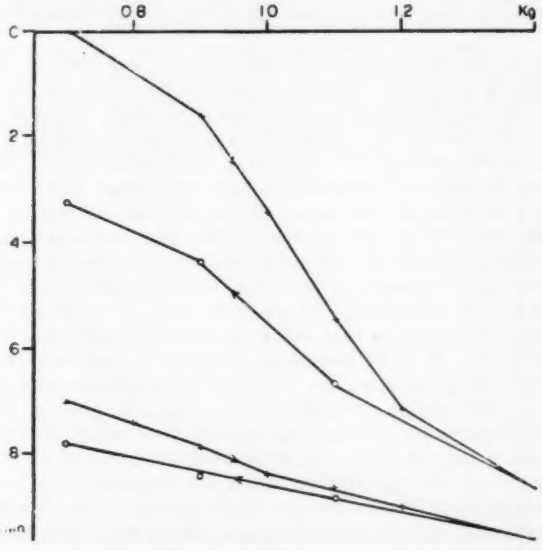


FIG. 1. — *Diagramas de tensión-longitud. Las dos curvas superiores corresponden a las cúspides de tétanos isotónicos y las dos inferiores al músculo en reposo. Las cruces fueron obtenidas aumentando la carga, como muestra la flecha; los círculos corresponden a los diagramas de regreso. Frecuencia de estimulación 70 por segundo.*

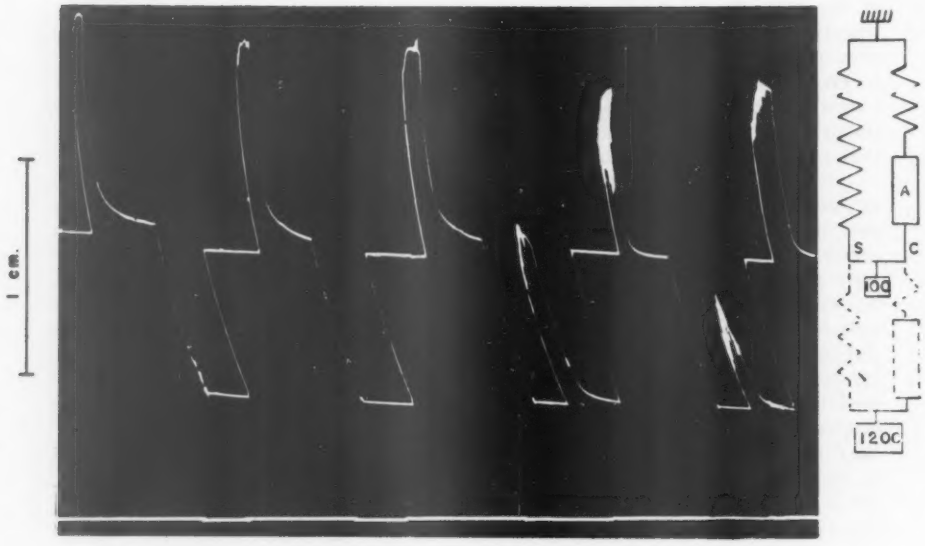


FIG. 2. — *Registros isotónicos originales que muestran el efecto del alargamiento y la contracción con cargas grandes sobre la magnitud de las respuestas con cargas pequeñas. Explicación en el texto. El dibujo de la derecha es un diagrama del modelo mecánico aceptado para el músculo.*

de un período de reposo se estiró el músculo con un peso de 1200 g sin estimular. Se quitó la carga y se volvió a estimular bajo la carga de 100 g (2ª respuesta). Se repitió el estiramiento y se volvió a estimular con 100 g (3ª respuesta). Al someter por tercera vez al músculo a la carga de 1200 g se estimuló con una frecuencia de 90 por seg (4ª respuesta). Se empleó esta frecuencia elevada con el propósito de que el acortamiento bajo esta carga fuera considerable. Se quitó la carga y después de un reposo de 15 minutos se volvió a estimular a 60 por segundo con 100 g (5ª respuesta). En este momento la anestesia era ligera y el animal se movía al estimular; de ahí la vibración observada en la respuesta. Un minuto después se colgaron al músculo 1200 gramos y se estimuló a 90 por segundo (6ª respuesta). Se quitó la carga y se administró más nembutal al animal y 30 minutos más tarde se estimuló nuevamente a 60 por segundo con 100 g. La 2ª y 3ª respuestas con 100 g son semejantes a la 1ª a pesar de los dos alargamientos. La 5ª y la 7ª respuestas son más pequeñas, debido a que se contrajo el músculo con la carga de 1200 g. Estas respuestas son de magnitud semejante, lo cual está de acuerdo con lo dicho en la sección anterior respecto a que el diagrama de tensión-longitud se repite. Los experimentos mostraron que la discrepancia entre las respuestas con una carga baja, antes de contraerse el músculo con la carga grande, y las obtenidas después, fué mayor cuanto más grande la carga y el acortamiento que sufrió el músculo bajo dicha carga.

C. *La influencia de la contracción con cargas grandes sobre el diagrama de tensión-longitud de reposo.* Es bien sabido que al alargar un músculo en reposo gradualmente y después soltarlo, regresa con longitudes mayores para las dife-

rentes cargas. La curva de regreso se repite si se vuelven a aumentar las cargas y después se vuelven a disminuir; esta repetición ocurre siempre que no se sobrepase la carga máxima aplicada al obtener el primer diagrama de tensión-longitud. La curva de las cruces de la figura 3 es un diagrama de tensión-longitud de reposo obtenido estirando primero al músculo con 800 g, después disminuyendo las cargas, y finalmente volviendo a aumentarlas. En seguida, estando sometido el músculo con la carga de 800 g, se estimuló con una frecuencia de 100 por seg. La cúspide de la respuesta que se obtuvo está representada por la cruz al final de la vertical trazada a esta carga. Obsérvese que la longitud mínima que adquirió el músculo al estimularlo fué menor que la longitud que tenía en reposo con la carga de 20 gramos. Después de esta estimulación el diagrama de tensión-longitud de reposo se modificó obteniéndose la curva de los círculos. Esta curva se recorrió cuatro veces sin cambios.

Discusión

I. Un modelo apropiado que se ha propuesto para los elementos elásticos del músculo ([2, 5]) está representado por el esquema que aparece en la figura 2. La rama *S* representa un elemento elástico pasivo en paralelo con la rama *C*, que incluye el componente contráctil (A), en serie con un elemento elástico pasivo. Las propiedades elásticas de los elementos pasivos son independientes de la actividad del sistema contráctil, es decir, que los sistemas pasivos siguen un diagrama de tensión-longitud invariable.

La discontinuidad que muestran en la parte superior las dos ramas del modelo de la figura 2, se hizo con el propósito de señalar que la longitud del modelo en la figura no es la del músculo con carga de 100 g. El extremo inferior del modelo con esta carga se hizo coincidir con la basal de 100 g del registro quimográfico. La parte inferior punteada representa el alargamiento que sufrió el músculo al colgarle 1200 g. Dos condiciones debe llenar el modelo. Las longitudes de ambas ramas deben ser en cualquier momento iguales. La tensión P_S en la rama *S* más la tensión P_C en la rama *C* debe ser siempre igual a la tensión registrada en los extremos del músculo.

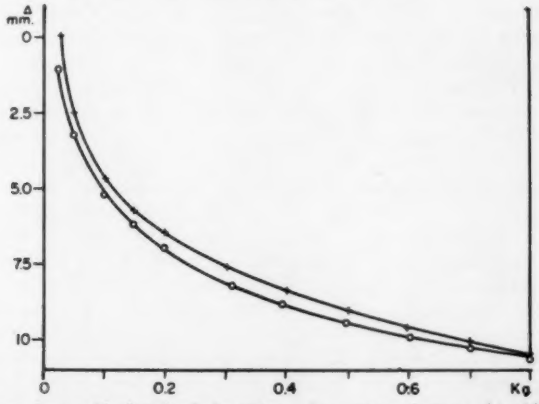


Fig. 3. — *Diagramas de tensión-longitud de reposo. Las cruces fueron obtenidas antes de que se contrajera el músculo con la carga de 800 gramos al ser estimulado a 100 por segundo. Los círculos fueron obtenidos después.*

La figura 2 muestra que al volver a estimular bajo la carga de 100 g después que el músculo se contrajo con 1200 g, la respuesta fué menor. Sugerimos que esta disminución es debida a que el número de elementos activos era más pequeño. Llamemos P'_S y P'_C a las tensiones en las ramas *S* y *C* cuando el músculo estaba estirado con 100 g. y llamemos P''_S y P''_C a las tensiones con la carga de 1200 g. Es claro que $P''_S > P'_S$. Cuando el músculo fué estimulado estando sometido a la carga de 1200 g, se acortó hasta adquirir una longitud mínima un poco menor que la que tenía en reposo con 100 g. Por lo tanto la tensión P_S disminuyó de P''_S a un valor un poco menor P'_S. En consecuencia la tensión P_C aumentó continuamente al irse acortando el músculo de tal manera que $P_C > P''_C$. El aumento de P_C puede ser tan considerable que al irse acortando el músculo, produce un rompimiento en alguna parte de la rama *C* de algunas fibras del músculo y, puesto que el aumento de P_C es continuo, los rompimientos de los distintos elementos deberían ir ocurriendo a diferentes niveles de la contracción. Al alcanzarse la cúspide de la respuesta el músculo cuenta con un número de elementos activos menor que el número con el que inició la contracción. El argumento implica que mientras más grande es el acortamiento, mayor es el incremento de P_C; por lo tanto el número de elementos que se rompen debe ser mayor. Esto está de acuerdo con lo señalado en la sección B con respecto a la discrepancia entre las respuestas con una carga baja antes de contraerse el músculo con una carga grande, y las obtenidas después. La discrepancia es más acentuada mientras más grande es la carga y mientras mayor el acortamiento del músculo con esta carga.

II. *La irreversibilidad de los diagramas de tensión-longitud tomados con cargas altas.* En la sección I se mostró que al contraerse el músculo con cargas grandes, la cúspide de la respuesta se alcanza con un número de elementos activos menor que el número con que se inició la contracción y que esta disminución es mayor al aumentar la carga. Esta inferencia está de acuerdo con lo señalado en la sección A respecto a la diferencia entre los diagramas de tensión-longitud de las cúspides de las respuestas (fig. 1); la diferencia se acentúa mientras mayor es la carga máxima aplicada.

De acuerdo con lo anterior, los diagramas de tensión-longitud comunicados por Hill ([5]), Wilkie ([11]) y Schottelius y Senay ([10]) mencionados en la introducción, pueden explicarse como sigue: la curva C de la figura 4 corresponde al diagrama de tensión-longitud de las cúspides de las respuestas isométricas de un músculo al ir aumentando la longitud. Supongamos que estando el músculo a la longitud L_1, se le estimula y la tensión en los extremos del músculo alcanza al valor P_1. Al alargar el músculo de L_1 a L_2 y estimular, el alargamiento y el aumento de tensión pueden ser suficientes para provocar el rompimiento de algunos elementos durante el desarrollo de la contracción; en lugar de alcanzarse la tensión P'_2 (punto en la curva C) sólo se alcanza la tensión P_2. Si se hubieran registrado regresos, éstos seguirían la curva C_1. Un alargamiento ulterior hasta L_3 provoca, al estimular, la ruptura de más elementos durante el desarrollo de la respuesta y sólo se alcanza la tensión P_3 en vez de P'_3. Los regresos a partir de L_3 darían la curva C_2. La prolongación en punteado de las curvas C, C_1 y C_2 corresponde a los valores longitud y tensión si no ocurriera ningún rompimiento de los elementos musculares. De hecho estas curvas sólo son definibles hasta los puntos (L_1, P_1), (L_2, P_2) y (L_3, P_3), respectivamente, y la unión de estos puntos por una curva corresponde a la comunicada por los autores arriba mencionados ([5, 10, 11]). En realidad esta curva no es un diagrama

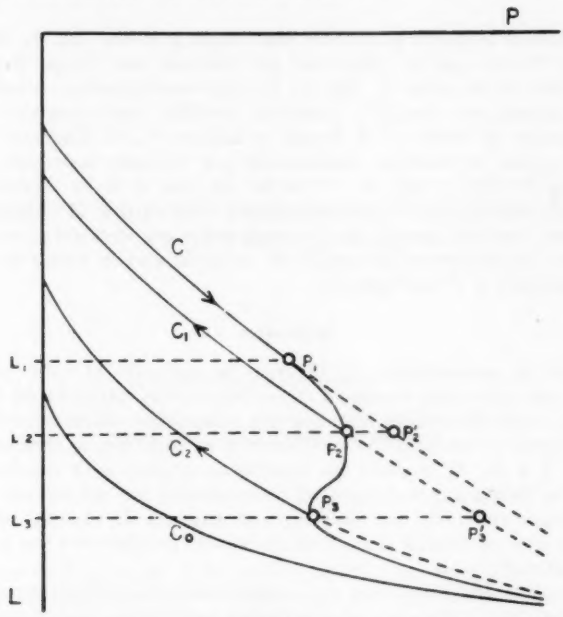


FIG. 4. — *Diagramas teóricos de tensión-longitud: C, C_1 y C_2 diagramas del músculo contraído isométricamente. C_0, diagrama de reposo. La curva que pasa por los puntos (L_1, P_1), (L_2, P_2) y (L_3, P_3) corresponde al diagrama de tensión-longitud propuesto por Hill* (5). *Véase la discusión.*

de tensión-longitud, puesto que es la unión de los puntos extremos de una serie de diagramas de tensión-longitud.

Un análisis semejante es aplicable a los resultados señalados en la sección A.

III. *La tensión en el componente contráctil durante el reposo.* Banus y Zetlin (1) comunicaron que la vaina de tejido conjuntivo del gastrocnemio de la rana daba un diagrama de tensión-longitud semejante al de reposo del músculo íntegro. Ramsey y Street (7) en fibras musculares únicas machacaron la parte central rompiendo el sarcoplasma en esa región y vieron que el diagrama de tensión-longitud de reposo de la fibra antes y después del machacamiento eran semejantes; concluyeron que el sarcolema es la estructura que determina la tensión de la fibra muscular en reposo. De acuerdo con lo expuesto por estos autores se ha supuesto que el componente contráctil en reposo no es elástico sino plástico y, por lo tanto, que a cualquier longitud su tensión es cero (6). Casella (4) repitió el experimento de Ramsey y Street, tomando el diagrama de tensión-longitud, no de toda la fibra, sino sólo de la región machacada y encontró que los diagramas de tensión-longitud antes y después del machacamiento eran muy diferentes y que, al contrario de lo indicado por Ramsey y Street, el sarcolema no ejerce una influencia apreciable en la tensión de reposo antes de ser estirado al 150 por ciento de su longitud con carga cero. Buchthal y Weis-Fogh (3), en músculo de insectos, encontraron resultados semejantes a los de Casella.

En la sección C se señaló que el diagrama de tensión-longitud de reposo se

modifica cuando el músculo se contrae con cargas grandes (fig. 3). En las secciones I y II se mostró que al contraerse un músculo con cargas grandes ocurre un rompimiento en la rama *C* (fig. 2) de algunos elementos musculares. Si la rama *C* en reposo no ejerciera ninguna tensión, como sugirieron Banus y Zetlin [1], Ramsey y Street [7] y Jewell y Wilkie [6], el diagrama de tensión-longitud de reposo no debería modificarse por haberse contraído el músculo con una carga de 800 g (fig. 3). El hecho de que se haya modificado dando mayores alargamientos para las mismas cargas, implica que al haberse debilitado la rama *C* una fracción mayor de la carga debe ser sostenida por la rama *S*. Estos hechos y su interpretación están de acuerdo con lo comunicado por Casella [4] y Buchthal y Weis-Fogh [3].

Resumen

Se estudió el gastrocnemio de la rata. Se estimuló el cabo periférico del nervio motor con estímulos máximos cuyas frecuencias variaron de 60 a 100 por segundo. Los resultados muestran que los diagramas de tensión-longitud del músculo en reposo y contraído se modifican si el músculo se contrae con cargas grandes (figs. 2 y 3). Al analizar los resultados se llega a la conclusión de que estos cambios se deben a que durante el acortamiento hay un aumento de tensión en el componente contráctil del músculo con ruptura de algunas fibras. Se concluye también que el sistema contráctil en reposo es elástico y no plástico como ha sido sugerido [6, 7].

Se sugiere que los diagramas de tensión-longitud atípicos encontrados por Hill [5] y otros [10, 11] para las contracciones isométricas son curvas que representan los puntos hasta los cuales son definibles una serie de diagramas de tensión-longitud con un número cada vez menor de fibras intactas (fig. 4).

El autor agradece al Dr. Juan José Mandoki el préstamo del equipo con que se realizó este estudio.

Summary

A study was made of the contraction of rat gastrocnemius muscle. The muscle was stimulated indirectly through its nerve at frequencies which ranged from 60 to 100 per sec. The results show that the tension-length diagrams of the muscle during rest and during contraction are modified if the muscle is contracting under heavy loads (figs. 2 and 3). It is assumed that during shortening there is an increase of the tension in the contractile elements of the muscle which leads to tearing of some fibres. Thus causing the changes mentioned above. This indicates that during rest the contractile component is elastic and not plastic as has been suggested [6, 7]. It is suggested that the tension-length for isometric contractions found by some authors [5, 10, 11] is a consequence of a progressive decrease in the number of intact muscle fibers as the length is increased (fig. 4).

Bibliografía

(1) Banus, M. G. y Zetlin, A. M.: *J. cell. comp. Physiol.*, 1938, *12*, 403.
(2) Buchthal, F., Kaiser, E. y Rosenfalk, P.: *Dan Biol. Medd.*, 1951, *21*, nº 7.
(3) Buchthal, F. y Weis-Fogh, T.: *Acta physiol. scand.*, 1956, *35*, 345.
(4) Casella, C.: *Acta physiol. scand.*, 1951, *21*, 380.
(5) Hill, A. V.: *Proc. roy. Soc. B.*, 1953, *141*, 104.
(6) Jewell, B. R. y Wilkie, D. R.: *J. Physiol.* (Lond.) 1958, *143*, 515.
(7) Ramsey, R. W. y Street, S. F.: *J. cell. comp. Physiol.*, 1940, *15*, 11.
(8) Rosenblueth, A., Alanís, J. y Rubio, R.: *Arch. int. Physiol.*, 1958, *66*, 354.
(9) Rubio, R.: ibíd., 1959.
(10) Schottelius, B. A. y Senay, L. C.: *Amer. J. Physiol.*, 1956, *186*, 127.
(11) Wilkie, D. R.: *J. Physiol.* (Lond.), 1956, *134*, 527.

THE EFFECTS OF ESTROGENS ON EXPERIMENTAL ATHEROSCLEROSIS

MANUEL RENÉ MALINOW (*)

(Institute of Physiology, Buenos Aires Medical School, University of Buenos Aires, Argentina.)

THE fact that estrogens are able to induce changes in genital tissues is very well known, but it is only recently that extragenital actions of estrogens have received greater attention, especially so in connection with the problem of atherosclerosis (**). A review of the pertinent extensive literature is out of the scope of the present paper and has been published elsewhere (1); only those observations pointing to the interrelationships existing between these hormones and atherogenesis (**) will briefly be mentioned here:

1) The different sexual distribution of atherosclerosis in the human being.

Early clinical studies had already shown that coronary atherosclerosis is by far more common in the male, a finding confirmed in large series of necropsies (2-4). That this sexual difference may be connected with functioning gonads is attested by the fact that it is greatest below 40 years of age and that it tends to disappear after the menopause. Nevertheless, it has also been claimed that the dissimilar sexual distribution of coronary atherosclerosis may partly be based on anatomical differences. Dock for instance (5), found in 24 newborn babies less than one day old, that the intima was thicker in males than in females; these findings, though can be related, not only with sex, but also with the presence of infection (6).

In fact, the problem is not as simple as stated because:

(*) Aided with Grants from the Asociación Argentina de Compañías de Seguros, Consejo Nacional de Investigaciones Científicas y Técnicas, the Asociación Cardiológica Inchauspe and with Grant H 3905, U.S. Public Health Service. Part of the present report was granted a Ciba Foundation Award on Research Relevant to the Problems of Ageing.

This paper summarises research conducted at the "Pabellón de Cardiología Inchauspe" in association with Amanda A. Pellegrino, Eugenia H. Ramos and David Hojman; work performed at the Institute of Physiology, Buenos Aires Medical School, was done with Grato E. Bur, Jaime A. Moguilevsky and Alicia Gutiérrez; research conducted in the Department of Physiology, State University of New York, was performed in association with Gertrude Lange.

(**) *ATHEROSCLEROSIS* designates in this paper a non-inflammatory arterial lesion, predominantly located in the intima of the arteries and showing lipid infiltration, cholesterol deposition and cellular proliferation, with or without necrosis or calcification; *ATHEROGENESIS*, development of atherosclerosis.

Received for publication, November 15, 1959.

a) the different sexual distribution of coronary atherosclerosis may disappear under two circumstances, at least: 1) in the negro female in which coronary disease starts earlier than in the negro male (7), and, 2) under the influence of diabetes (8).

b) in other arterial territories, sexual differences may not be maintained or can even be reversed. For instance, in a clinical and anatomical study performed on 1000 subjects up to 100 years af age, it was shown that calcification of the abdominal aorta and of the femoral arteries was more frequent in female (9); perhaps some of these cases were due to medial calcification and not to atherosclerosis.

2) The influence of gonadectomy and of estrogen administration upon the development of atherosclerosis in men and in women.

Two very well documented papers have shown the importance of oophorectomy and of estrogens on the development of atherosclerosis. The first paper, from the Mayo Clinic, compares the post-mortem findings in 49 women subjected to bilateral oophorectomy before the age of 45 and with a follow up of at least two years. Findings showed that the operated women had more atherosclerosis than 600 similarly studied control females, but less than 600 control men (10).

A second related report also suggests that estrogen therapy may decrease coronary atherosclerosis in men, as well as in other vascular territories in women: men with prostatic carcinoma and treated with large doses of estrogens for at least three months, showed less coronary atherosclerosis than patiens under smaller dosage of hormones or receiving no such therapy; in the same groups, no differences were found in other vascular territories. Women with breast carcinoma and under estrogenic therapy had less atherosclerosis in the aorta as well as in the coronary and cerebral arteries, than bilaterally oophorectomized patiens (11).

Finally, it might be quoted here, that patients with cirrhosis of the liver have less atherosclerosis than control subjects (12). It has been suggested that this fact may be explained by the well known decreased inactivation of estrogens in this liver condition (12).

3) The effect of estrogens in experimental atherosclerosis.

Stemming from an early observation that increased blood levels of calcium, phosphorus and fats are observed in the chicken during ovulation (13), it was shown that injected estrogens may reproduce these findings (14). Later on, prolonged hypercholesterolemia produced in cockerels by pellet implantation of diethylstilbestrol resulting in aortic atherosclerosis was reported (15). A totally different finding was described afterwards by Pick et al. (16), namely, the prevention and, then, the regression of coronary but not of aortic atherosclerosis in cholesterol-fed chickens under estradiol therapy (17). These observations have since, been amply confirmed (18). Recently, prevention of aortic atherosclerosis by other estrogenic substances has been reported in the chicken (19) and evidences extending estrogenic protection to other arterial territories has been presented (20). Differences in the action of several estrogenic hormones on atherogenesis have been demonstrated and it has been thus rightly suggested, that individual substances used in experiments should not be designed under the generic name of estrogens (19). Furthermore, androgens too, are able to prevent atherosclerosis in cholesterol-fed birds (21).

In other species, findings are not totally similar to those described in the chicken. In the rabbit, for instance, estradiol benzoate does not induce hypercholesterolemia nor aortic atherosclerosis (22), while aortic atherosclerosis is reduced by estradiol benzoate in the female but not in the male cholesterol-fed animal; similar actions are shared by androgens (23). Castration prevents these estrogenic effects (24), although other authors have reported a contrary finding, namely, that castration reduces atherosclerosis in cholesterol-fed female rabbits (25). Finally, as a further difference with the chicken, the several estrogenic hormone which have been tried, have not been able to prevent coronary atherosclerosis in cholesterol-fed rabbits (26).

A single paper on the effects of estrogens in rats shows that estradiol benzoate may initially increase and then reduce coronary lesions (27). The arterial lesions as defined in this report include medial sudanophilia; observations were confined to the coronary vessels.

4) Blood changes produced by estrogens.

Blood lipids are greatly increased in most of the experimentally induced atherosclerosis; consequently possible blood changes brought about by estrogens have been studied with great care. In spite of the fact that in the normal human being, blood cholesterol levels are similar in both sexes (28), it seems possible that in certain circumstances estrogenic hormones may regulate blood lipids. Thus, injection of several estrogens in the human being decreases blood cholesterol, increases phospholipids and shifts lipoproteins from the β-fraction to the α-fraction (29-31), and similar changes have been described in atherosclerotic patients (32).

Observations in animals do not reproduce these clinical findings. Female rats, for instance, have higher blood cholesterol levels than males and cholesterolemia shows a clear cyclic change,

higher levels being attained during estrus and lower ones during diestrus (33). When rats are placed under atherogenic diets, estrogens further increase blood cholesterol levels (34); similarly, in cholesterol-fed rabbits, females show a higher cholesterolemia and a greater variability than males (35).

Intimate mechanisms involved in the extragenital effects of estrogens have not been elucidated. Furthermore, the data already quoted fail to explain why some species react differently toward the atherogenic preventing effects of estrogens, nor why these hormones may act predominantly on certain arterial territories. In an effort to answer some of these points and, at the same time, to pose new questions, the present work was initiated aiming at:

1) extending the study of the effect of estrogens on experimental atherosclerosis in different territories of several species, and,

2) correlating the vascular effects with endocrine changes.

As our results seemed to indicate that estrogens may exert a local action on atherosclerotic vessels, a study was made on:

3) the influence of local factors on the development of atherosclerosis,

4) the distribution of radioactive estrogens in atherosclerotic animals, and,

5) the modification of the aorta by estradiol in vitro.

1

Effects of estrogens on experimental atherosclerosis

1 a) Effects of estrogens on coronary and aortic atherosclerosis in cholesterol-fed chickens (*).

The effects of estradiol benzoate administration on atherogenesis was studied in cholesterol-fed cockerels.

METHODS. The methods have been published elsewhere (17) and only the main features will be mentioned here. Ninety-five 1 day old Hy-line cockerels were received from a commercial hatchery and reared in a battery brooder. They were fed commercial chick starter mash until they were 7 weeks of age. They were then divided into several groups, but only the four groups pertaining to this experiment will be described here:

Group 1 (control A) received mash supplemented with 2 % cholesterol and 5 % cotton seed oil. Group 2 (experimental A) received the same diet plus daily injections of 1 mg estradiol benzoate (Progynon B, Schering); from these two groups, chicks were sacrificed at 12, 15 and 20 weeks of age. Group 3 (control B), received the diet for 13 weeks. Group 4 (experimental B) received the same diet for 13 weeks and for the last 5 weeks of this period, in addition, a daily injection of 1 mg of estradiol benzoate; the last 2 groups were sacrificed at 20 weeks of age.

At sacrifice, the heart and great vessels of 5 birds of each group were fixed in 10 % formaldehide, placed in bottles, labelled with a code number and sent to us to be studied "blindly". The heart vas cut one mm below the a-v grove and 28 consecutive sections studied with several histochemical techniques (**). All arteries greater than 50μ were counted, and sudanophilia or proliferation of the intima noted. The aortas were cut into 3 segments (except those from group 2 which were cut into 2 segments), and each segment classified with the naked eye as positive or negative for atherosclerosis.

(*) The anatomical studies were performed in association with D. Hojman and A. A. Pellegrino on material published previously (17) obtained through the kindness of Dr. Louis N. Katz.

(*) Hematoxylin-eosin; elastic fibers with Gallego's (Bol. Soc. Esp. Biol. 1924, *11*); fats with Sudan IV; cholesterol with Lieberman-Bouchard's (Comp. Rend. Acad. Sc. 1927, *184*, 1206) and Windaus' (Bull. d'Histol. Appl. 1926, *3*, 316); calcium with Kossa's (Cowdry, E. V. Laboratory technique in Biology and Medicine. The Williams and Wilkins Co., Baltimore 1948, page 5); connective tissue with Van Giesson's (Cowdry, loc cit. page 258); glucoproteins with McManus' (Nature 1946, *158*, 202): mucopolysaccharides with Hale's (Arch. Path. 1951, *52*, 189).

TABLE I

Effect of estradiol benzoate on coronary atherosclerosis in cholesterol-fed chickens

	Group	# of arteries	Arterial diameter ≥ 150 μ % intimal changes Sudanophilia (a)	proliferation (b)	# of arteries	Arterial diameter 50-149 μ % intimal changes Sudanophilia (c)	proliferation (d)
Experimental procedure (*) ..							
Cholesterol (control A)	1	308	64.6	15.2	320	77.8	8.7
Cholest-estradiol benzoate (**) (prevention)	2	141	10.6	2.1	189	15.4	2.6
Cholesterol (control B)	3	234	54.2	13.6	288	56.9	3.8
Cholest-estradiol benzoate (**) (regression)	4	218	28.4	4.1	332	18.0	0
"t" (***)	a		1 vs 2 14.594 $p < 0.001$			3 vs 4 5.920 $p < 0.001$	
	b		1 vs 2 5.874 $p < 0.001$			3 vs 4 3.878 $p < 0.001$	
	c		1 vs 2 17.309 $p < 0.001$			3 vs 4 10.788 $p < 0.001$	
	d		1 vs 2 3.521 $p < 0.001$			3 vs 4 0.682 $p > 0.40$	

(*) See text.
(**) Estradiol benzoate, 1 mg. intramuscularly/day.
(***) "t" Student's test. Throughout this paper, the statistical significance of results has been determined with the aid of Fisher's tables (51).

TABLE II

Effect of estradiol benzoate on aortic atherosclerosis in cholesterol-fed chickens (*)

	Group	Number of aortic segments	% of macroscopic atherosclerosis
Cholesterol (control A)	1	15	60
Cholesterol and estradiol benzoate (prevention)	2	10	40
Cholesterol (control B)	3	15	33
Cholesterol and estradiol benzoate (regression)	4	15	40
"t"	1 vs 2	1.174	$p > 0.3$
"t"	3 vs 4	0.702	$p > 0.5$

(*) See footnote of table I.

RESULTS. (Table I an II). As can be seen in table I, coronary sudanophilia as well as endothelial proliferation are decreased in a statistically significant way, in both experimental groups under estradiol benzoate therapy when arteries $\geq$ 150 μ in diameter are considered. The same is true with arteries between 50 and 149 μ, except in relation with proliferation, where the differences are not statistically significant because of the small number of proliferated vessels observed in the cholesterol-fed control group. No differences were found in the aortas.

COMMENTS. Although grading of the lesions was performed using a totally different procedure from the one used by the Chicago group, the results found by us duplicate their conclusions, namely, that estradiol benzoate is able to prevent and to induce regression of coronary atherosclerosis in cholesterol-fed chickens without influencing aortic lesions.

1 b) Effects of estrogens on spontaneous atherosclerosis in the rat (*).

Previous reports on experimentally-induced atherosclerosis were not designed to determine whether estrogens also prevent spontaneous atherosclerosis. Consequently, a study was made of the effects of these hormones on spontaneous atherosclerosis in the rat. It must be added parenthetically that, in spite of previous negative reports, we were able to demonstrate the occurrence of spontaneous atherosclerosis in the rat ([36]), findings which were lately confirmed ([37]).

METHODS. Male white William strain rats, betwen 15 and 24 months old, were separated into 2 groups: 1) 30 controls, and, 2) 10 experimental animals receiving intraperitoneally 5-10 μg of estradiol benzoate (17 β-estradiol, 3 benzoate) daily except Sundays for periods up to 60 days. The methods of study have been previously reported ([38]) and only the main features will be described here. Upon sacrificing the animals, the organs were fixed in 10 % formaldehide and 20 lengthwise aortic, 60 cardiac and 60 kidney frozen sections were stained with hematoxylin-Sudan IV. In all arteries greater than 30 μ and clearly showing the different wall structures, lesions were graded from 0.5 to 3.0 accordingly with the amount of intimal sudanophilia and/or proliferation (for details see 38). Results were quantitated by multiplying this grading times an index accordingly with the extension and the number of lesions found in each organ.

(*) These studies were performed in association with A. A. Pellegrino and have been published elsewhere ([38]).

RESULTS. (See Table III). As can be seen in Table III, control rats show a slight degree of atheromatosis, while none of the estradiol benzoate – treated animals had arterial lesions.

COMMENTS. Although the actual incidence of atherosclerosis in the control rats is minimal, the fact that none was demonstrated in the injected group suggests that estradiol benzoate prevents or decreases the spontaneous lesions in all the studied territories; furthermore this work also demonstrates that the hormone may exert a protective action upon other vessels than the coronary arteries.

1 c) Effects of estrogens on experimentally induced atherosclerosis in the rat (*).

Having thus demonstrated that estradiol benzoate is able to decrease spontaneous atherosclerosis in rats, it was deemed of interest to see if the hormone could be effective on experimentally-induced atherosclerosis in the same species.

METHODS. The methods have been published elsewhere and only the main features will be mentioned here (38). Male white William strain rats, between 15 and 24 month old and kept on a standard laboratory diet were used. Unilateral cellophan perinephritis was performed under a clean but not sterile technique; the animals also received 5 ml of sunflower seed oil (I.N. 130) by gastric gavage daily except sundays. Two groups of animals were studied: 1) 40 animales as described, and, 2) 18 rats as above, plus 5-10 μg of estradiol benzoate intraperitoneally, 6 times a week, for periods up to 60 days. Histological procedures and anatomical grading as described under 1 b).

RESULTS. (See Table IV). By comparing Tables 3 and 4, it can be seen that cellophan perinephritis and forced oil-feeding increased the severity of atherosclerosis, especially so in the coronary arteries. The administration of estradiol benzoate was only partially able to counteract for this increased atherogenic stimulus: although no atherosclerosis was found in the aorta nor in the proximal branches such as the intercostal, mesenteric, etc., the hormone showed no protective action in the coronary nor in the renal vessels.

COMMENTS. This experiment suggests that estradiol benzoate, even when preventing atherosclerosis in some vascular territories, may not be effective in others if the atherogenic stimulus is too pronounced. Such was the case with the coronary and the renal arteries which were spared of spontaneous atherosclerosis but not of the experimentally-induced one. It is possible that these two conditions may not be the same, but it seems evident that, with the low doses used by us, the anti-atherogenic action proved to be different in both cases. Furthermore, using higher dosis of estradiol benzoate, Moskowitz et al. (27) have shown that the hormone may protect the coronary arteries of rats placed under atherogenic conditions.

1 d) Effects of estrogens on the arteries of cholesterol-fed rabbits (**).

Since it has been demonstrated already that estrogens are able to influence atherosclerosis in chickens and in rats, it seemed worth while studying its effects on rabbits. Reports in the literature show that even a high dosage of estrogens is ineffective in male rabbits (23), while contradictory conclusions have been reached in female rabbits (24, 25) (***).

(*) These studies were performed in association with A. A. Pellegrino and have been reported elsewhere (38).

(**) These studies were performed in association with A. A. Pellegrino and E. H. Ramos and have been partly reported elsewhere (39).

(***) It might be of interest to remember in this connection that rabbits metabolize estrogens in a very peculiar way since they are able to excrete these hormones as the 17-α-isomer of estradiol, which is almost inactive as an estrogenic hormone (40).

TABLE III

Effects of estradiol benzoate on spontaneous atherosclerosis in rats

Group	Atherosclerosis												
	Aorta				Aortic branches			Coronaries			Renal arteries		
	# of rats	# of rats	%	Grade	# of rats	%	Grade	# of rats	%	Grade	# of rats	%	Grade
Control	30	1	3	0.08	4	13	0.32	10	33	0.48	3	10	0.10
Estradiol benzoate	10	0	0	0	0	0	0	0	0	0	0	0	0

Modified from Malinow, M. R. and Pellegrino, A. A.: Arch. Path., 1958, *65*, 47.

TABLE IV

Effects of estradiol benzoate on atherosclerosis of perinephritic rats

Group	Atherosclerosis												
	Aorta				Renal arteries			Coronaries			Aortic branches		
	# of rats	# of rats	%	Grade	# of rats	%	Grade	# of rats	%	Grade	# of rats	%	Grade
Control (cellophan perinephritis)	40	2	5	0.10	8	20	0.56	15	37	1.98	4	10	0.27
Cellophan perinephritis estradiol benzoate	18	0	0	0	0	0	0	5	27	1.94	3	16	0.41

Modified from Malinow, M. R. and Pellegrino, A. A.: Arch. Path., 1958, *65*, 47.

METHODS. The methods have been published elsewhere and only a summary will be made here (39). Male rabbits of mixed breed weighing 1.5 to 2.5 Kg were maintained on a standard laboratory diet. All animals except the controls, also received 6 days a week 750 mg of crystalline cholesterol in 5 ml of sunflower seed oil (I.N. 130) thoroughly mixed with mashed carrots. One week after starting the cholesterol feeding, half of the animals thus fed were injected intravenously twice a week with 4 ml of saline in 2 divided doses, 2 hours apart; the other half received intravenously twice a week, 2 ml of 6% Dextran (R) and 0.5 mg of microcrystallized estradiol benzoate (17 β-estradiol, 3 benzoate) in 5 ml of saline, also in divided doses, 2 hours apart. Microcrystals less than 1 μ in diameter and suitable for intravenous injections were freshly prepared by precipitating a concentrated alcoholic solution of estradiol benzoate into normal saline.

The controls and 43 experimental animals which survived 60 days on this regime, were sacrificed and their aortas examined. The observed macroscopic atherosclerosis was graded on an arbitrary 0 to 4 plus scale. Differences existing with the published report are due to the fact that 3 rabbits which presumably did not eat the special diet and had normal blood cholesterol levels at sacrifice, were deleted.

RESULTS. (Tables V to VIII). Results shown in Tables V to VIII demonstrate that the Dextran (R)-estrogen-treated group had less atherosclerosis than the saline treated group in the aorta and in the renal arteries, while the coronary arteries had a similar involvement in both groups. Table VIII shows that blood cholesterol levels at sacrifice, were somewhat reduced in the estrogen-treated group, but the differences were not statistically significant at the 5% level.

COMMENTS. This experiment suggests that intravenous microcrystals of estradiol benzoate following the injection of Dextran (R) are able to prevent aortic and renal atherosclerosis in rabbits, but not the coronary involvement. Prevention, then, has been limited to some of the explored territories while others have not been influenced. Results obtained in the aorta as reported by others conflict with our own findings; this may be explained by assuming:

a) that the protection is afforded through blood cholesterol changes. It is difficult to accept this possibility because the difference among the cholesterol-fed groups is not statistically significant and also because it could not account for the protection seen in some territories and not in the coronary arteries. Nevertheless, these blood lipid changes, well confirmed in the literature, may partly contribute to the vascular effects of estrogens.

b) That the protection is afforded because of the intravenous administration. The hormone has been used so far, per os or intramuscularly with negative results and the differences with the present experiment should be furtherly explored. It has been suggested that microcrystals are incorporated through phagocytosis by the arterial endothelium and could become veritable pellets with a predominant action within the arterial wall (39). In this connection, incorporation of colloidal thorium in the altered endothelia of atheromatous plaques has been demonstrated to occur in cholesterol-fed rabbits (41).

c) That the protection is afforded because of the previous action of Dextran (R). Dextran (R) itself, though, is not able to influence atherogenesis (see below) but it could possibly influence estrogen metabolism by blocking the reticulo-endothelial system (42).

TABLE V

Effects of estradiol benzoate on aortic macroscopic atherosclerosis in cholesterol-fed rabbits (*)

Group	# of rabbits (**)	With lesions		Without lesions		"t"	p
		Number	%	Number	%		
Cholesterol-saline	19	5	26.3	14	73.7	3.074	< 0.01
Cholesterol-Dextran (R) estradiol benzoate ..	21	13	61.8	8	38.2		

(*) See footnote of Table I.
(**) Animals with cholesterolemia above control levels.
Taken from Malinow, M. R., Pellegrino, A. A. and Ramos, E. H.: Proc. Soc. exp. Biol. N. Y., 1958, *97*, 446.

TABLE VI

Effects of estradiol benzoate on coronary atherosclerosis in cholesterol-fed rabbits (*)

Group	Coronary atherosclerosis			
	± of rabbits (**)	Grade (***)	"t"	p
Cholesterol-saline	19	16.5 ± 11.73	0.887	0.4
Cholesterol-Dextran(R-estradiol benzoate ...	21	20.3 ± 15.0		

(*) See footnote of Table I.
(**) Animals with cholesterolemia above control levels.
(***) Mean ± standard deviation.

TABLE VII

Effects of estradiol benzoate on renal arterial lesions in cholesterol-fed rabbits (*)

Group	# of rabbits (**)	Renal arterial lesions grade (***)	"t"	p
Cholesterol-saline	19	1.18 ± 2.1	2.130	< 0.05
Cholesterol-Dextran(R)-estradiol benzoate	21	0.14 ∓ 0.2		

(*) See footnote of Table I.
(**) Animals with blood cholesterol levels above control levels.
(***) Mean ± standard deviation.

TABLE VIII

Effects of estradiol benzoate on plasma cholesterol levels in cholesterol-fed rabbits (*)

Group	# of rabbits	Plasma cholesterol mg/100 ml	"t"	p
Control	10	52.2 ± 20.0 (**)		
Cholesterol-saline	19	659.6 ± 383.5		
Cholesterol-Dextran(R)-estradiol benzoate	20	480.2 ± 268.9	1.765	0.10 > p > 0.05

(*) See footnote of Table I.
(**) Mean ± standard deviation.

2

ENDOCRINE ASPECTS OF ESTROGENIC ADMINISTRATION TO NORMAL AND ATHEROSCLEROTIC ANIMALS

In order to investigate the possible interdependence of the endocrine and the vascular actions of estrogens, and, at the same time, the possible influence of the route of administration, the following experiments were performed:

2 a) Relationship between endocrine effects and vascular action of estrogens in atherosclerotic animals (*).

In animals subjected to atherogenic stimuli, the effects of estradiol benzoate on the weight of testes, adrenals and hypophysis was studied. These endocrine glands were chosen because administration of estrogens is associated with an atrophy of testes in males and in some species with hypertrophy of the other two glands (43).

METHODS. A) Rats. The testicles of the rats described under 1 c were studied. After removal, the organs were weighed and fixed in 10 % formaldehide; one testicle was selected at random in each animal, transversely cut and 4 frozen sections stained with hematoxylin-Sudan IV. Testicular function was roughly graded as follows: 0, no spermatozoids seen; 1, these cells were seen in less than half of the examined tubules; 2, spermatozoids were present in half of the examined tubules; 3, more than half of the tubules showed spermatozoids; 4, all tubules showed spermatozoids. In the final interpretation testicles graded 0 though 1 were considered as atrophic and those grades 3 or 4 as normal (see details in 38).

B) Rabbits. The testicles, adrenals and hypophysis of the rabbits described under 1 d, were carefully dissected and weighed immediately upon removal.

The endocrine changes were correlated with the anatomical findings in the arteries of both kind of animals.

RESULTS. (Table IX and X). Results shown in Table IX indicate that estradiol benzoate at the dosage used, did not increase the percentage of atrophic testicles in unoperated rats while the incidence of atherosclerosis was reduced to zero. This suggests that in rats, it is possible to separate the endocrine from the vascular effects. Furthermore, the hormone was able to induce an atrophy on the testicles of perinephritic rats (**) which had an increased incidence of atherosclerosis. The experimental circumstances were obviously not similar to those present in unoperated rats since the atherogenic stimulus was probably increased; nevertheless, this experiment shows that estrogens given in amounts able to induce atrophy of the testicles may be unable to prevent atherosclerosis.

Table X shows that a similar independence between vascular and endocrine effects may be present in the rabbit. It is seen here that in cholesterol-fed rabbits, weight of the testicles and of the hypophysis was not altered by injecting estradiol benzoate, while the adrenals showed the expected hypertrophy under cholesterol feeding, but no further difference was brought about by estrogen administration.

COMMENTS. These experiments suggest that the endocrine and the vascular actions can be separated, since atherosclerosis was prevented in two species without inducing further weight changes in the endocrines studied. It may thus be concluded that although castration reduces atherosclerosis in men (44) and although estrogens can induce testicular atrophy and prevent atherosclerosis,

(*) These experiments were performed in association with A. A. Pellegrino and E. H. Ramos and have been reported elsewhere (38, 39).

(**) The perinephritic rats weighed less than the controls and this may explain why the same dosage of estradiol was now able to atrophy the testes.

TABLE IX

Comparison of arterial and testicular lesions of normal and atherosclerotic rats

Group(*)	# of rats	Aorta	Aortic branches	Coronary arteries	Renal arteries	# of rats	% with normal testicles
Intact controls	30	0.08	0.32	0.48	0.10	36	72
Estradiol benzoate	10	0	0	0	0	9	77
Cellophan perinephritis	40	0.10	0.56	1.98	0.27	26	84
Cellophan perinephritis + estradiol benzoate	18	0	0	1.94	0.41	17	6

(*) See text.
Modified from Malinow, M. R. and Pellegrino, A. A.: Arch. Path., 1958, *65*, 47.

TABLE X

Weight of endocrines in cholesterol-fed rabbits (*)

Group	# of rabbits	Testicles (g)	# of rabbits	Hypophysis (mg)	# of rabbits	Adrenals (mg)
(1) Control	11	4.582 ± 1.678 (**)	9	21.5 ± 5.1	9	0.478 ± 0.102
(2) Cholesterol-saline	20	4.227 ± 1.805	15	22.8 ± 7.2	15	0.879 ± 0.499
(3) Cholesterol-Dextran (R)-estradiol benzoate	21	3.986 ± 1.152	16	21.3 ± 7.6	16	1.053 ± 0.296
"t" 1 vs 2		0.538 $p < 0.6$		0.522 $p < 0.6$		2.356 $p < 0.05$
"t" 1 vs 3		1.185 $p < 0.3$		0.073 $p < 0.9$		5.727 $p < 0.001$
"t" 2 vs 3		0.507 $p < 0.7$		0.572 $p < 0.9$		1.170 $p < 0.3$

(*) See footnote of Table I.
(**) Mean ± standard deviation.
Taken from Malinow, M. R., Pellegrino, A. A. and Ramos, E. H.: Proc. Soc. exp. Biol. N. Y., 1958, *97*, 446.

these hormones might influence arterial lesions through some unknown mechanism, independently of its hypotrophic action on the testicles.

2 b) Comparison of the endocrine effects of estradiol administered as microcrystals and as oil solution (***).

The negative results obtained by other authors administering estradiol in conventional preparations to male cholesterol-fed rabbits (22, 23), contrasted with the preventive action reported above when estradiol benzoate was given as intravenous microcrystals. This prompted us to study the effects of different preparations of estradiol on the weight of several endocrines, including the testes, seminal vesicles, adrenals and hypophysis. Although in this experiment, estradiol and not estradiol benzoate was used as before, comparison of these compounds is valid because the ester is hydrolyzed in the body and the overall endocrine actions are very similar (45).

METHODS. Forty eight young male rats of the Williams strain were divided into four groups of 12 animals each; each animal received 75 μg of estradiol twice a week during 4 weeks, as follows: a) intravenous microcrystals; b) intramuscular microcrystals, and, c) intramuscular oil solution. Twelve further animals not receiving any medication were taken as controls. The rats were weighed at the beginning and at the end of the experiment; upon being sacrificed with ether, the testicles, the empty left seminal vesicle, the adrenals and the hypophysis were dissected free and weighed immediately upon removal.

RESULTS. As it is seen in Table XI, 75 μg of intravenous or intramuscular estradiol microcrystals were without effect on the testis, seminal vesicles and the hypophysis, while the same preparation given as intramuscular oil solution was able to atrophy the testicles and the seminal vesicles while increasing the weight of the hypophysis and of the adrenals.

COMMENTS. Intravenous and intramuscular microcrystals of estradiol, at the dosage used, proved to have less estrogenic effect than the hormone in oil solution. On the other hand, we have seen that intravenous injections of microcrystals were able to prevent atherosclerosis in rabbits without inducing endocrine weight changes. If, as already shown, prevention of atherosclerosis is also effected in species other than the rabbit, intravenous microcrystals could be of value when it is necessary to maintain the estrogenic effects as low as possible.

2 c) Injection of intravenous estradiol benzoate microcrystals in the human being (*).

Because intravenous estradiol microcrystals have less endocrine effects in the male rat and rabbit than conventional hormonal therapy, the feasibility of its administration in the human being was explored.

METHODS. The methods have been reported elsewhere (46) and only the main features will be mentioned here. 500 μg of freshly prepared estradiol benzoate microcrystals in 5 ml of saline were given intravenously to three patients with unoperable carcinoma. The absence of any untoward reaction prompted us to give a total of 32 injections to 20 male patients. The hormone was given at dosages ranging from 50 to 500 μg; immediate estrogenic effects were investigated in the first morning urinary specimen during several days by observing in the fresh sediment the presence of desquamated iodophilic cells from the prostatic utriculum, which in the male, is subjected to similar estrogenic influences as the vaginal cells are in the female (47). The patients were observed clinically and signs of untoward effects noted.

(***) These experiment have been performed in association with J. A. Moguilevsky and A. Gutiérrez, and will be reported elsewhere.

(*) These experiments have been performed in association with G. E. Bur and reported elsewhere (46).

TABLE XI

Weight of endocrines in rats under estrogenic therapy (estradiol 75 μg twice a week during 4 weeks)

Group	Nº of animals	Testicles g/100 g of body weight	Left empty seminal vesicle mg/100 g of body weight	Adrenals mg/100 g of body weight	Hypophysis mg/100 g of body weight
CONTROLS	12	14,219 ± 0.497 (**)	1,690 ± 0,338	1,886 ± 0,020	3,517 ± 0,207
INTRAVENOUS ESTRADIOL MICROCRISTALS	12	13,599 ± 0,394	1,111 ± 0,207	2,194 ± 0,120	3,484 ± 0,196
INTRAMUSCULAR ESTRADIOL MICROCRISTALS	12	13,339 ± 0,452	1,357 ± 0,260	2,260 ± 0,100	3,222 ± 0,160
INTRAMUSCULAR ESTRADIOL IN OIL SOLUTION	12	10,902 ± 0,160	0,431 ± 0,111	2,638 ± 0,091	4,326 ± 0,269
"t" 1 vs 2		0,981 $p < 0.4$	1,496 $p < 0.2$	2,545 $p < 0.02$	0,116 $p > 0.9$
"t" 1 vs 3		1,313 $p < 0.3$	0,785 $p < 0.5$	3,703 $p < 0.01$	1,134 $p < 0.3$
"t" 1 vs 4		6.391 $p < 0.001$	3,692 $p < 0.01$	8,173 $p < 0.001$	2,444 $p < 0.05$
"t" 3 vs 4		5,196 $p < 0.001$	3,211 $p < 0.01$	2,820 $p < 0.01$	3,558 $p < 0.01$

(*) See footnote Table 1.
(**) Mean ± standard deviation of the mean.
Taken from Moguilevsky, J. A., Gutiérrez, A. and Malinow, M. R.: Rev. Soc. argent. Biol., 1959, *35*, 103.

TABLE XII

Estrogenic changes in the urinary sediment brought about by intravenous estradiol benzoate microcrystals in the human being

Dose (μg)	Number of injections	Estrogenic effect (*)
50	3	1
100	6	1
200	17	13
300	3	3
500	3	3

(*) See text.
Taken from Malinow, M. R. and Bur, G. E.: Ciencia e Invest., 1957, *13*, 519.

RESULTS. (Table XII). As can bee seen in Table XII, 50 to 100 μg of intravenous estradiol benzoate microcrystals only occasionally induced a positive estrogenic action, while doses of 200 μg and above, regularly did show such an effect. This action was seen starting the following day from the injection and lasted for a few days.

COMMENTS. The intravenous injection of estradiol benzoate microcrystals was well tolerated in the human being and no untoward effects were noted. As shown above, when given to animals, its estrogenic action was less marked than when administered in the conventional way. In the human being, small doses of this preparation, generally had no estrogenic effects discernible with our methods. Since estrogens are given to man as a therapy for atherosclerosis, this new preparation seems to be worth trying.

3

THE IMPORTANCE OF LOCAL FACTORS IN THE DEVELOPMENT AND IN THE PREVENTION OF ATHEROSCLEROSIS (*)

Current ideas stress the changes that blood lipids undergo under estrogen therapy and try to explain the vascular action through such lipid changes. Such an explanation, though, fails to account for the protection afforded to some but not all vessels in the same animal.

In view of the fact that steroid hormones, and, also because prevention of atherogenesis can not be correlated with a generalized estrogenic effect, it seemed possible that estradiol may modify certain unknown local factors in the arteries, and through such a mechanism, be able to prevent atherosclerosis. We thus tried to demonstrate the importance of local factors in the development of atherosclerosis. These local factors, including filtration, reabsorption, synthesis as well as transformation as defined elsewhere (49), have been approached indirectly and considered together.

METHODS. The methods have been published elsewhere (48), and only the main features will be considered here. Sixty-two male rabbits, 1.6 to 2.3 kg of weight on a standard laboratory diet, were weighed weekly (these animals are those remaining from a larger initial group; animals which died before being sacrificed have been omitted). Forty eight animals were given in addition, 1 g of crystalline cholesterol, daily except Sundays, in 5 ml of sunflower seed oil

(*) These experiments were performed in association with A. A. Pellegrino and E. H. Ramos and have been published elsewhere (48).

(I. N. 130) thoroughly mixed with mashed carrots. From this group and twice a week, 15 rabbits were given intravenously 0.2 mg of estradiol benzoate microcrystals; 11, 2 ml of 6 % Dextran (R) and 9, the same amounts of Dextran (R) and the hormone, two hours apart. Note that in previous experiments (cf 1 d), the amount of cholesterol given to the animals was smaller and the dose of estradiol benzoate more than doubled the one used here. The results which were obtained in the present experimental animals were similar in all respects; consequently, and for the purposes to be reported here, this experiment may be considered to consist of 14 control and 48 cholesterol-fed rabbits.

After 90 days, the animals were anesthetized with pentobarbital, blood was withdrawn from the aorta for cholesterol determinations, and a complete autopsy performed. The lesions in the aorta were drawn on a paper and "blindly" graded from 0 to 4 plus, accordingly with the extent and thickness of the lesions. The heart was fixed in 10 % formaldehide, cut into six slices parallel to the a-v groove and sectioned at 10 μ; in each segment, 10 random frozen sections were stained with hematoxylin-Sudan IV, and all arteries greater than 30 μ and clearly showing the different wall structures, graded from 0.6 to 3.0 accordingly with the amount of lipid infiltration and cellular proliferation of the intima. The individual lesions were multiplied by an index taking into account the number and the extent of the lesions (for details consult 48). Cholesterol levels were determined in plasma obtained upon sacrificing the animals with the method of Bloor (50); the cholesterol content of the aorta was determined in an alcoholic extract after 8-10 hours of continuous boiling. The observed parameters were then correlated with the "r" method and its statistical significance determined with the use of tables (51).

TABLE XIII

Chemical and anatomical correlations in control and in cholesterol-fed rabbits. Values of the correlation coefficient "r" (*)

	Aortic cholesterol	Coronary atherosclerosis	Aortic atherosclerosis
Blood cholesterol levels	0.55 (**)	0.79 (**)	0.70 (**)
Aortic atherosclerosis	0.91 (**)	0.58 (**)	—

(*) See footnote of Table I.
(**) Statistically significant at the level of $p < 0.01$.
Taken from Malinow, M. R., Pellegrino, A. A. and Ramos, E. H.: Acta physiol. lat.-amer., 1958, *8*, 37.

RESULTS. (Table XIII and figures 1 to 3). The calculated coefficients "r" between blood cholesterol, aortic cholesterol content, aortic macroscopic atherosclerosis and coronary microscopic atherosclerosis, although they must be considered to be only a first approximation because of discontinuity in the anatomical gradings, show a positive correlation between all observed parameters. When the results are plotted graphically (fig. 1 to 3), it is seen that a correlation is also evident, in spite of great scattering of the individual results.

COMMENTS. These results confirm many previous experiments showing that in the rabbit hypercholesteremia, increased cholesterol content of the aortic wall and generalized atherosclerosis follow cholesterol feeding. Nevertheless, although these parameters are mutually interdependent, they are not very closely correlated. In other words, deposition of cholesterol into the arterial wall is only partly dependent on blood cholesterol levels, and local factors not clearly defined at the present, but which must include reabsorption from the arterial wall as well as filtration, local synthesis and local transformation, should be taken into account. These mechanisms, then, can explain scattering of the observations when the studied parameters are correlated with blood cholesterol

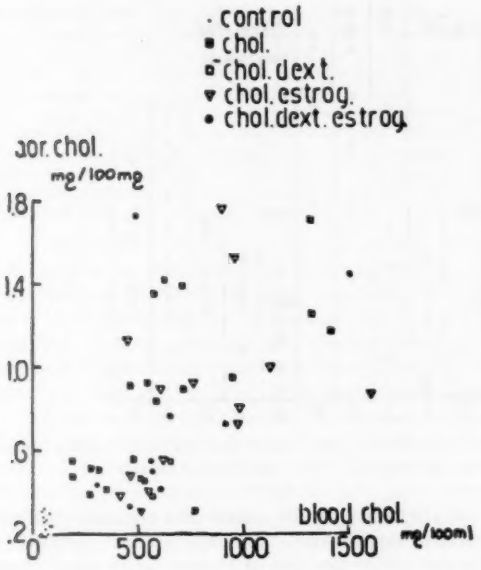


FIG. 1.—*Aortic cholesterol content against blood cholesterol levels. All cholesterol-fed rabbits are randomly distributed. Chol., cholesterol, dext., Dextran (R); estrog., 17 β-estradiol benzoate. Discussed in text (Fig. 1 through 3, taken from Malinow, M. R., Pellegrino, A. A. and Ramos, E. H. Acta Physiol. Latinoamer. 8, 1958, 37).*

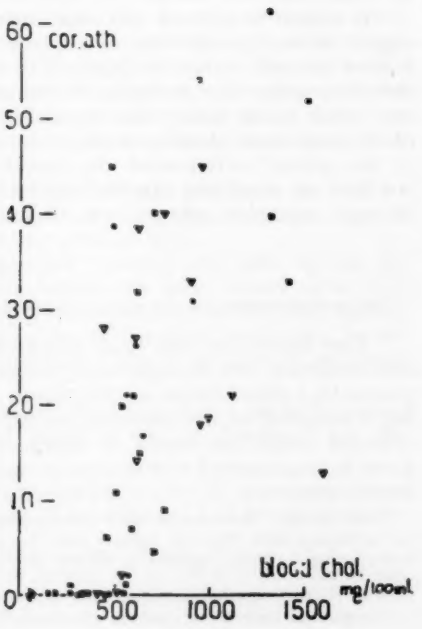


FIG. 2.—*Coronary atherosclerosis against cholesterol levels in cholesterol-fed rabbits. Symbols as before.*

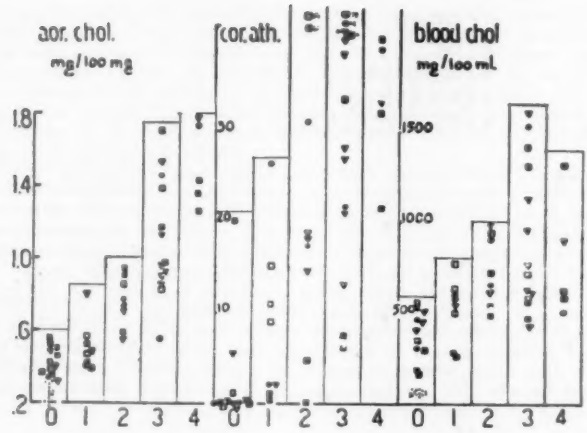


FIG. 3. — *Aortic cholesterol content, coronary atherosclerosis and blood cholesterol levels against macroscopic atherosclerosis.* Discussed in text. Symbols as before.

levels. Finally, the lack of a strict parallelism between aortic and coronary atherosclerosis further suggest some different mechanisms controlling the reactions of these two vessels. Our ignorance in the understanding of factors other than blood cholesterol which might influence atherogenesis has been clearly pointed out by Weinhouse and Hirsch (52) as well as by McMillan et al. (53).

It should be stressed that experiments of the kind described here, can only suggest several possibilities to explain the observed facts, but a more direct type of research should be planned to account for these findings. Nevertheless, the observations are theoretically compatible with the presence of arterial factors which could modify the deposition of cholesterol with a certain independence from blood cholesterol levels and which could modify as well the reaction of the arterial wall toward the deposited cholesterol. Such a hypothesis does not deny the possibility that the arterial factors themselves may also be influenced through endocrine, physical, etc., factors as well as by blood cholesterol levels.

4

THE DISTRIBUTION OF RADIOACTIVE ESTRADIOL IN ATHEROSCLEROTIC ANIMALS (*)

The indication that local factors may contribute to the development of atherosclerosis, and the suggestions advanced that estrogens may influence atherogenesis by a direct action on the vessels, led us to study the distribution of radioactive estradiol in atherosclerotic animals in order to determine if the hormone is found within the vessels; of course, the demonstration that an injected hormone is concentrated within certain tissues does not prove that it exerts a local action there.

METHODS. The methods have been presented elsewhere and only the main features will be mentioned here (54). Six rabbits were fed 1-2 % cholesterol thoroughly mixed with their food during a 60-day period; 2 control animals were kept on a standard laboratory diet.

(*) These experiments have been performed in association with A. A. Pellegrino and G. Lange, and have been reported elsewhere (53).

Estradiol 6,7 H 3 (**) with 17 β-estradiol as a carrier was prepared as microcrystals suitable for intravenous injections (cf. supra); each animal received approximately 25 μcu and 1 mg of the carrier as follows: 4 cholesterol-fed and 2 control rabbits received intravenously 5 ml of Dextran (R) in an attempt to partially block the reticulo-endothelial system; 2 hours later the hormone was given intraperitoneally to 2, and intravenously to an additional 2 cholesterol-fed rabbits, while the 2 controls received the estradiol intravenously; the 2 remaining cholesterol-fed animals were given the microcrystals intravenously without the Dextran (R). The animals were sacrificed 3 hours after the intravenous and 5 hours after the intraperitoneal injections. Several organs including the ascending aorta, heart, spleen, liver, lung, intestine and adrenal glands were removed and fixed in 10% formaldehide; radioautographs were then performed with the stripping film technique (*) and developed 3 months later.

RESULTS. (Figure 4 and 5). Aorta. In all animals, radioactive material was irregularly distributed in granules which were more numerous in the outer two-thirds of the arterial wall, especially around the vasa vasorum. The inner third also showed scattered reduced areas but endothelial cells only occasionally exhibited active material. In one cholesterol-fed animal which had received estradiol intravenously and Dextran (R), an atheromatous plaque, which was especially studied, showed a pronunced accumulation of radioactivity.

Heart and coronary vessels. In animals, tagged material was present especially within the capillaries of the inner third of the myocardium. No activity was detected within the coronary arteries except in one case where it was seen in the media of a medium sized vessel (this animal had received estradiol intravenously and no Dextran (R).

Other tissues: Lungs. A very slight radioactivity was present within the lungs but no consistent pattern could be described. In one case, active material was detected in the media of a pulmonary artery.

Kidney. A heavy deposit of tagged material was seen in all cases; maximal positivity was found in the cortical as well as in the inner medullary regions. In the cortical regions, radioactivity was present in the parietal layer of Bowman's capsule, in the cavity of the capsule and in the cavity of a few convoluted tubules; granules were also seen in the free border as well as within the epithelial cells of the convoluted tubules. Radioactivity was also observed in the intertubular connective tissue. In only one instance were reduced granules seen in a renal artery.

Intestine. Radioactivity was detected within the muscular layer, the external border of the intestinal cylindrical epithelium, and in the connective stem of the villi; no deposits were found within the arterial walls.

Testicles. Heavy depositions of radioactive material were seen in the germinal epithelium, apparently concentrated within the more mature cells; only scanty granules were observed in the interstitium and none within the arteries.

Liver: Focal distributions of a few granules were seen in some cases. In one instance, radioactivity was present in a biliary duct and in another, in the media of a portal vein.

Adrenal glands. Tagged material was not observed in the parenchymatous cells but in one instance it was present within a few capillaries of the glomerular zone.

COMMENTS. The present line of investigation is limited in certain aspects because radioactivity found, after injection of estradiol 6, 7 H 3, within tissues

(**) Obtained through the New England Nuclear Corp., Boston, Mass.

(*) The technique was kindly demonstrated to once of us (M. R. M.) by Dr. Patrick J. Fitzgerald, who also generously provided us with the necessary films (A. R. 10 Kodak stripping films).

FIG. 4. — *Radioautography of the aorta in a cholesterol-fed rabbit injected with estradiol 6.7 H_3. Observe the black granules showing radioactive material in the adventitia and the outer third of the media, probably distributed within and around capillaries; radioactive material is also present in an atheromatous plaque at the top. (X 150 Taken from Malinow, M. R., Pellegrino, A. A. and Lange, G., Acta Endocr. 1959, 31, 500.)*

which have been fixed in 10 % formaldehide, merely indicates the presence of the hormone and/or some water-insoluble metabolites. The presence of small amounts does not preclude its biological activity since the sites of localization of a hormone after injection may not be related to its physiological action. Furthermore, if the turnover of the hormone is high, a pronounced biological activity could still be exercised in spite of low concentrations of the tagged material.

In spite of these limitations, certain conclusions can be tentatively advanced: estradiol and/or hormonal metabolites was found in the normal and the atheromatous aorta of rabbits suggesting a dual distribution through the vasa vasorum and through the intima. This accords with well known laws of circulation of fluids within the arterial wall (49). Normal endothelial cells only occasionally showed radioactivity whereas in one instance, an atheromatous plaque greatly concentrated the hormone and/or its metabolites. In connection with the problem of atherosclerosis, then, the present observations suggest that estrogens could exert some local action in the aorta of the rabbit, and specially in atheromatous plaques; if such a conclusion be true, vessels *could* be con-

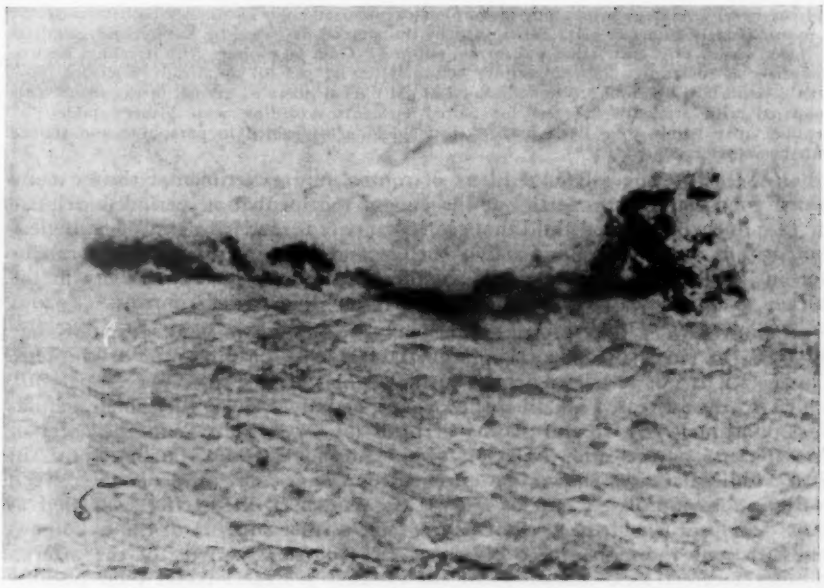


FIG. 5. — *A higher magnification of the plaque from Fig. 4 (X 420)* (Same as Fig. 4).

sidered to be target organs for the estrogenic hormones, but of course, more experiments are needed in this respect before accepting such a conclusion.

The fact that the hormone and/or its metabolites was not uniformly distributed within the aorta and that minimal or no radioactivity was seen in other arterial territoires, could be due to differences in affinities or to some other unknown factors, thus pointing out the importance of local mechanisms in the biology of arteries.

5

"IN VITRO" EFFECTS OF ESTRADIOL ON THE AORTA OF CHICKENS

The experiments already reported suggest that estrogenic hormones, besides modifying blood lipid levels ([29, 31]), may prevent atherosclerosis through a direct action on the vessels themselves. Nevertheless, a direct action of hormones on the arteries has not been reported, with the exception of the studies of Werthessen ([55, 57]). In the present experiment it is demonstrated that estradiol may enhance "in vitro" the activity of an inespecific phosphomonoesterase in the aorta of chickens.

MATERIAL AND METHODS. The aorta of 10 white leghorn cockerels, weighing between 500 and 1000 g, was removed under sterile conditions, cut into 10 to 12 rings of approximate 2 mm in length and incubated during 72 hs at 37.5 ± 1° C in modified roller tubes with a balanced saline medium ([6]) into which 30 % of bovine serum, 0.05 % of ethanol as well as streptomycin and penicilin were incorporated; aortic rings were also incubated with the same medium except that 2 μg of 17 β-estradiol were added per ml. Throughout the experiment, pH was maintained by changing the medium as required. At the end of the experimental period, the tissues were blotted with filter paper and weighed; they were then frozen

and cut in 10 μ sections with a microtome, homogenized and the alkaline phosphatase activity in approximately 20 mg of aorta determined by the method described by Kaplan and Narahara for blood serum [58]. Incubation was carried out at 37° C for 30 minutes with disodium phenylphosphate as substrate and with sodium borate buffer at pH 9.4 as already described [59]. Results performed by duplicate and expressed as μM phenol liberated per mg of wet tissue, were compared with Student's "t" test for paired elements according with Fisher's tables [51]. Representative tissues were fixed in 10 % formaldehide, embedded in paraphine and stained with hematoxilin-eosin (*) (**).

RESULTS. The cells and fibers of control and experimental tissues maintained good staining properties at the end of the incubation period; a relative increase in the interstitial fluid though, was apparent rendering thus meaningless, comparisons of the enzymatic activity before and after incubation. As it can be seen in Table XIV, estradiol increased the alkaline phosphatase activity in the chicken aorta when compared with the corresponding paired controls.

COMMENTS. The present experiments demonstrate that estradiol may enhance "in vitro" the alkaline phosphatase activity of the aorta of chickens. This action is not restricted to the cardiovascular tissues since it is apparently similar to the increased alkaline phosphatase activity seen in the female genital tract of mamals [60], in the plasma of birds [61, 62], as well as in the aorta of chickens and of rats after injection of the hormone [62, 63]. Furthermore, "in vitro" studies have also shown that estrogenic hormones may influence the oxygen consumption of isolated systems [64, 66]. Consequently, these effects, as well as those demonstrated in the present paper, lend further support to the hypothesis that estrogenic hormones may influence the metabolic activity of the arteries through a direct local action. Estrogens in such a respect could then possibly behave similarly as in the uterus, vagina, mamma and skin where they show effects when locally applied [67].

TABLE XIV

Modification of alkaline phosphatase activity in vitro. *Results expressed in μM of phenol/mg of wet chicken aorta liberated under stated conditions* (*)

Group	Number of animals	With added steroid 2 μg/ml	Paired controls	Difference	"t"	p
17 β-estradiol	10	0.136 ± 0.022	0.045 ± 0.014	0.090 ± 0.014	6.500	< 0.001

(*) Determinations performed by duplicate. Average ± standard error.

The significance of our findings in connection with enhancement of aortic atherosclerosis as seen in birds after diethylstilbestrol administration [15] or with prevention and regression of coronary atherosclerosis in cholesterol-fed cockerels after estrogenic therapy [16, 18], remains to be clarified. It seems appropiate, though, to quote the cautious words of Hechter, before undue emphasis be paid to results obtained in isolated systems: "*in vitro* reactions with hormones may be completely unrelated... to the nature of essential underlying cellular mechanisms involved in hormone response" [68]. Nevertheless, the fact that injection of estradiol increases alkaline phosphatase activity in the aorta of

(*) We are thankful to Dr. Grato E. Bur who kindly reviewed the sections.

(**) These experiments are going to be reported more fully elsewhere (Circulat. Res., in press).

chickens and of rats ([61, 62]), and that similar results are seen "in vitro", apparently demonstrates that estrogens *may* influence arterial metabolism by modifying local enzymatic mechanisms. Much more work in this field, though, is clearly needed before such modified local mechanisms could be linked with atherogenesis.

Summary and conclusions

1) Observations pointing to the interrelationship existing between estrogens and the development of atherosclerosis have been shortly reviewed and stated as follows: a) coronary atherosclerosis is by far more common in male than in female patients, and this difference tends to disappear after the menopause; b) bilaterally oophorectomized women have more atherosclerosis than suitable controls; c) male patients with prostatic carcinoma receiving large doses of estrogens have less atherosclerosis than patients without such a therapy; d) some estrogenic hormones are able to prevent and to effect a regression of coronary atherosclerosis in cholesterol-fed chickens, and, d) estrogens are able to "feminize" the blood lipid pattern as found in the human male.

2) Experiments reported here show that: a) estradiol benzoate when given either prophylactically or therapeutically, prevented or induced the regression of coronary atherosclerosis in cholesterol-fed chickens; aortic atherosclerosis was not affected. This confirmed previous findings in the literature; b) rats given estradiol benzoate (contrarywise to the controls), showed no signs of spontaneous atherosclerosis in the aorta, the proximal aortic branches, the coronary and the renal arteries; it thus seemed that the hormone may exert a protective action upon vessels other than the coronary arteries. Experimental findings have so far stressed the action of estrogens only upon the coronary vessels; c) rats with cellophan perinephritis showed more atherosclerosis than their controls; if those operated animals received estradiol benzoate, no atherosclerosis was found in the aorta nor in the proximal aortic branches while the lesions were not reduced in the coronary nor in the renal vessels. This seems to show that the hormone may not be effective in some vascular territories if the atherogenic stimulus is too pronounced; d) in cholesterol-fed male rabbits injected with estradiol benzoate given as intravenous microcrystals, atherosclerosis was prevented in the aorta and the renal arteries, but no protection was afforded to the coronary vessels. Since other authors have not been able to show such an effect, the possible role of the method of administration was further explored.

3) Although a thorough study of the endocrine factors involved was considered to be out of the scope of the present work, the following experiments related with the problem of atherosclerosis were performed: a) in animals subjected to atherogenic stimuli and under estrogenic therapy, the weight of the testes, adrenals and hypophysis was determined and findings correlated with the presence of atherosclerosis. It was thus shown that in male rats, prevention of atherosclerosis was not correlated with an induced atrophy of the testes. In rabbits, prevention of aortic and renal atherosclerosis by estrogens was observed without changes in the weight of the explored endocrines as compared with suitable controls; b) as a sideline of the present work it seemed of interest to compare the action of estradiol microcrystals administered intravenously and intramuscularly with that of a conventional oil solution of the same hormone. Results showed that estradiol microcrystals had less endocrine effects in male rats when given intravenously than when administered intramuscularly. Furthermore, in the conditions tested, intramuscular estradiol in oil

solution was more effective in inducing endocrine weight changes than the microcrystalline intramuscular injection; c) finally, estradiol benzoate microcrystals were injected in men without untoward effects.

4) Since prevention of atherosclerosis by estrogens could not be correlated with a generalized effect on the weight of the endocrines and considering that steroid hormones may be able to act locally on certain structures, it was deemed of interest to study the importance of local factors in the development of atherosclerosis. Results showed that hypercholesterolemia, increased cholesterol content of the aortic wall and generalized atherosclerosis followed cholesterol feeding in rabbits. Nevertheless, although these parameters were mutually interdependent, they were not closely correlated. Such findings suggested, then, that besides blood cholesterol levels, some local factors not clearly defined at the present time, may influence deposition of cholesterol into the arterial wall and its atherogenic reaction.

5) Radioactive estradiol (estradiol 6,7 H3) was injected into cholesterol-fed and into normal rabbits. Radioautographies showed that the hormone (and/or its metabolites) was distributed within the aortic walls as well as in other organs; an atheromatous plaque which was especially studied, showed a pronounced accumulation of radioactivity. Although the significance of this finding is open to question, the possibility that estradiol might exert a local action on the arteries and on the atheromatous plaques can be entertained.

6) Finally aortas of chickens were incubated during 72 hours in the presence of estradiol and the alkaline phosphatase activity determined. The enzyme was enhanced by the hormone when compared with suitable paired controls. Consequently, estrogens have been able to influence arterial metabolism by modifying local enzymatic mechanisms. Much more work in this field, though, is clearly needed before such modified local mechanisms could be linked with atherogenesis.

References

(1) Malinow, M. R.: *Ciencia e Invest.*, 1956, *12*, 395.
(2) Clawson, B. J. and Bell, E. T.: *Arch Path.*, 1949, *48*, 105.
(3) White, N. K., Edwards, J. E. and Dry, T. J.: *Circulation*, 1950, *1*, 645.
(4) Ackerman, R. F., Dry, T. J. and Edwards, J. E.: *Circulation*, 1950, *1*, 1345.
(5) Dock, W.: *J. Amer. med. Ass.*, 1946, *131*, 875.
(6) Minkowsky, W. L.: *Amer. J. med Sci.*, 1947, *214*, 623.
(7) McNay, *L. M.* (Jr.) and Keil, P. G.: *Arch. intern. Med.*, 1955, *96*, 762.
(8) Ricketts, H. T.: *Amer. J. Med.*, 1955, *19*, 933.
(9) Goldbloom, A. A., Eiber, H. B., Boyd, L. J., Chapman, I., Deutscheberg, O. and Loewe, W. R.: Sciemtific Exhibits, *Arch. intern. Med.*, 1956, *97*, 359.
(10) Wuest, J. H. (Jr.), Dry, T. J. and Edwards, J. E.: *Circulation*, 1953, *7*, 801.
(11) Rivin, A. V. and Dimitroff, S. P.: *Circulation*, 1954, *9*, 533.
(12) Hall, E. M., Olsen, A. Y. and Davis, F. E.: *Amer. J. Path*, 1953, *29*, 993.
Creed, D. L., Baird, W. F. and Fisher, E. R.: *Amer. J. med. Sci.*, 1955, *230*, 385.
(13) Riddle, O.: *Trans. Amer. Philos. Soc.*, 1927, *66*, 497, quoted by Fleishman, W. and Fried, I. A.: *Endocrinology*, 1945, *36*, 406.
(14) Lorenz, F. W., Chaikoff, I. L. and Enteman, C. C.: *J. biol. Chem.*, 1938, *126*, 763.
(15) Lindsay, S., Lorenz, F. W., Enteman, C. and Chaikoff, I. L.: *Proc. Soc. exp. Biol.* N. Y., 1946, *62*, 315.
Lindsay, S., Nichols, C. W. (Jr.) and Chaikoff, I. L.: *Arch. Path.*, 1955, *59*, 173.
(16) Pick, R., Stamler, J., Rodbard, S. and Katz, L. N.: *Circulation*, 1951, *4*, 468 (P).
(17) Pick, R., Stamler, J., Rodbard, S. and Katz, L. N.: *Circulation*, 1952, *6*, 276.
(18) Katz, L. N. and Stamler, J.: *Experimental Atherosclerosis*, C. C. Thomas, Springfield, Ill., 1953.
(19) Cembrano, J., Penna, A., Vial, M. and Mardones, J.: *Proc. First Sc. Meeting*, Asoc. Latinoamer. Ciencias Fisiológ., Punta del Este, Uruguay, April 24-27, 1957, p. 52.

(20) KUROYANAGI, T., RODBARD, S. and WILLIAMS, C.: *Circulation*, 1957, *16*, 501 (P).
(21) COOK, D. L., EDGREN, R. A. and HARRIS, T. W.: *Circulat. Res.*, 1957, *5*, 54.
(22) LUDDEN, J. B., BRUGER, M. and WRIGHT, I. S.: *Endocrinology*, 1941, *28*, 999.
(23) LUDDEN, J. B., BRUGER, M. and WRIGHT, I. S.: *Arch. Path.*, 1942, *33*, 58.
(24) BRUGGER, M., WRIGHT, I. S. and WILAND, J.: *Arch. Path.*, 1943, *36*, 612.
(25) MININNI, G., CONTRO, S. and CHECHIA, C.: *Circulat. Res.*, 1955, *3*, 191.
(26) STAMLER, J., PICK, R. and KATZ, L. N.: *Circulation*, 1953, *8*, 436 (P).
(27) MOSKOWITZ, M. S., MOSKOWITZ,A. A., BRADFORD, W. L. (JR.) and WISSLER, R. W.: *Arch. Path.*, 1956, *61*, 245.
(28) DEUOL, H. J. (JR.): *The lipids, Their chemistry and their biochemistry*. Interscience Pub. Inc., New York 1955, 2, p. 400.
PETERS, J. P. and VAN SLYKE, D. D.: *Quantitative Clinical Chemistry*. Williams & Wilkins Co., Baltimore 1946, 2nd. ed., p. 476.
(29) EILERT, M. L.: *Amer. Heart J.*, 1949, *38*, 472.
(30) TOWNSEND, E. W., PERRY, J. W. and ROEN, P. B.: *Circulation*, 1952, *6*, 478.
(31) BARR, D. P., RUSS, E. M. and EDER, H. A.: *Circulation*, 1952, *6*, 455.
(32) RUSS, E. M., EDER, H. A. and BARR, D. P.: *Amer. J. Med.*, 1955, *19*, 4.
(33) FILLIOS, L. C., KAPLAN, R., MARTIN, R. S. and STARE, F. J.: *Amer. J. Physiol.*, 1958, *193*, 47.
(34) PRIEST, R. E., SCHROEDER, M. T., RASMUSSEN, R. and WISSLER, R. W.: *Proc. Soc. exp. Biol.* N. Y., 1957, *96*, 298.
(35) FILLIOS, L. C. and MANN, G. V.: *Circulat. Res.*, 1956, *4*, 406.
(36) MALINOW, M. R. and PELLEGRINO, A. A.: *Arch. Path.*, 1956, *61*, 11.
(37) HUMPHREYS, E. M.: *Quart. J. exp. Physiol.*, 1957, *42*, 96.
(38) MALINOW, M. R. and PELLEGRINO, A. A.: *Arch. Path.*, 1958, *65*, 47.
(39) MALINOW, M. R., PELLEGRINO, A. A. and RAMOS, E. H.: *Proc. Soc. exp. Biol.* N. Y., 1958, *97*, 446.
(40) HEARD, R. D. H.: *Recent Progr. Hormone Res.*, 1949, *4*, 25.
(41) DUFF, G. L., MACMILLAN, G. C. and LAUTSCH, E. V.: *Amer. J. Path.*, 1954, *30*, 941.
(42) Unpublished observations.
(43) MORELL, J. A. and HART, G. W.: *Endocrinology*, 1941, *29*, 995.
(44) BARR, D. P., RUSS, E. M. and EDER, H. A.: *Trans. Ass. Amer. Phycns.*, 1952, *65*, 102, quoted by MILLER, D. C., STARE, F. J. and WHITE, P. D.: *Amer. J. med. Sci.*, 1956, *232*, 329.
(45) SEGALOFF, A.: *Recent Progr. Hormone Res.*, 1949, *4*, 85.
(46) MALINOW, M. R. and BUR, G. E.: *Ciencia e Invest.*, 1957, *13*, 519.
(47) GIL VERNET, S.: *Patología Urogenital*, Ed. Paz Montalvo, Madrid, 1953, 2, vol. I, p. 165.
(48) MALINOW, M. R., PELLEGRINO, A. A. and RAMOS, E. H.: *Acta physiol. lat.-amer.*, 1958, *8*, 37.
(49) MALINOW, M. R., HOJMAN, D. and PELLEGRINO, A. A.: In "World Trends in Cardiology", *1, Cardiovascular Epidemiology, A. Keys and White, P. D.*, ed., Hoeber-Harper, New York, 1956, p. 126.
(50) BLOOR, W. R. and KNUDSON, A.: *J. biol. Chem.*, 1916, *27*, 107.
(51) FISHER, R. A. and YATES, F.: *Statistical Tables for biological, agricultural and medical Research*. Hafner Publishing Co., Inc., 5th ed., New York, 1957.
(52) WEINHOUSE, S. and HIRSCH, E. F.: *Arch. Path.*, 1940, *30*, 856.
(53) MCMILLAN, G. G., HORLICK, L. and DUFF, G. L.: *Arch. Path.*, 1955, *59*, 285.
(54) MALINOW, M. R., PELLEGRINO, A. A. and LANGE, G.: *Acta Endocr.*, 1959, *31*, 500.
(55) WERTHESSEN, N. T., NYMAN, M. A., HOLMAN, R. L. and STRONG, J. P.: *Circulat. Res.*, 1956, *4*, 586.
(56) WERTHESSEN, N. T.: *Circulat. Res.*, 1958, *6*, 759.
(57) WERTHESSEN, N. T.: In *Hormones and Atherosclerosis*, ed. by G. Pincus, Academic Press, Inc., Pub., New York, 1959, Page 131.
(58) MALINOW, M. R., FERNÁNDEZ, M. A., GIMENO, A. L. and BUR, G. E.: *Nature*, 1959, *183*, 1262.
(60) ALKINSON, W. B. and ELFTMAN, H.: *Endocrinology*, 1947, *40*, 30.
(61) LANDAUER, W., PFEIFFER, P. A., GARDNER, W. V. and SHAW, J. C.: *Endocrinology*, 1941, *28*, 458.
(62) MALINOW, M. R., GIMENO, A. L., PELLEGRINO-IRALDI, A. A., FERNÁNDEZ-GIMENO, M. A., LACUARA, J. L. and MOGUILEVSKY, J. A.: *Nature* (in press).
(63) Unpublished.
(64) HAYANO, M., SCHILLER, S. and DORFMAN, R. I.: *Endocrinology*, 1950, *46*, 387.
(65) GUIDRY, M. A., SEGALOFF, A. and ALTSCHUL, A. M.: *Endocrinology*, 1952, *50*, 29.
(66) ROBERTS, S. and SZEGO, C. M.: *J. biol. Chem.*, 1953, *201*, 21.
(67) SPEERT, H.: *Physiol. Rev.*, 1948, *28*, 23.
(68) HECHTER, O.: *Vitam. and Horm.*, 1955, *13*, 293.

THE INFLUENCE OF THE NECK TOURNIQUET ON THE BLOOD ANTIDIURETIC POTENCY OF RATS

L. BARNAFI, S. VIAL and H. CROXATTO

(Laboratorio de Fisiología, Universidad Católica de Chile.)

DIFFERENT results have been reported by various authors in the study of the antidiuretic potency of the serum or plasma of normal rat (Birnie, 1949 and 1950; Dicker, 1950; Ames, 1952; Ginsburg, 1953; and Giere, 1954). Some of these discrepancies can be attributed to different types of anesthesia. Others, to an increase of antidiuretic activity occuring during haemorrhage, due to vasopressin discharge from the hypophysis (Ginsburg, 1953). In order to test the last possibility, a method for collecting blood samples with the exclusion of head circulation, seemed necessary.

The present paper is concerned with experiments in rats where blood was obtained by aortic puncture after a tourniquet was placed on their necks.

METHODS

Blood was obtained from adult rats (200-250 gm body weight), anesthetized with Avertin, Winthrop Chemical Co., Inc. (25 mg Avertin and 12.5 mg Amylene Hydrate per 100 gm body weight), intraperitoneally. The animal was bled through the aorta immediately after the neck was crushed with the tourniquet. No anticoagulant was used. The blood samples were injected in test animals, in groups of four for each donor (0.5 and 1.0 ml per 100 gm body weight), intraperitoneally, according to the method described by Burns (1931). Saline (0.9 % NaCl) was injected in control groups. Pooled urinary specimens were obtained at 30 minutes intervals.

Received for publication, October 2nd, 1959.

Results

In the first place, a comparison between the antidiuretic potency of the blood of normal rats, bled and with a tourniquet, and that of another group of rats, without tourniquet was made. The samples obtained from both groups showed antidiuretic activity, if compared to saline controls. However, this activity was higher in the animals bled without a tourniquet. (See Table I). In another series of experiments, the antidiuretic potency of the blood from normal and adrenalectomized rats (15-30 days), was studied. In both groups, the tourniquet was used. Blood of adrenalectomized rats showed a greater urinary inhibition than that of normal animals. No difference was observed in the blood of normal animals receiving 1 % NaCl, compared to those drinking water.

TABLE I

Cumulative percentage of water load excreted (*)

	Nº animals	Blood injected in ml	Minutes after blood injection			
			30	60	90	120
Rats with tourniquet	59	0.5	12.0 ± 1.1	47.1 ± 1.9	67.3 ± 1.9	74.8 ± 1.6
Rats without tourniquet ..	20	0.5	5.0 ± 1.4	30.4 ± 3.3	61.5 ± 3.0	75.0 ± 2.4
Rats with tourniquet	13	1.0	9.1 ± 2.4	45.1 ± 5.1	58.6 ± 4.3	62.3 ± 5.0
Rats without tourniquet ..	6	1.0	0.2 ± 0.3	19.4 ± 3.0	35.2 ± 1.4	38.5 ± 1.8
Rats with tourniquet, drinking 1 % NaCl:						
Normal	20	0.5	14.7 ± 1.7	52.0 ± 2.8	70.0 ± 2.0	75.8 ± 1.5
Adrenalectomized	17	0.5	4.6 ± 1.5	27.8 ± 3.6	49.7 ± 3.4	56.7 ± 3.2
Saline controls	40	0.5-1.0	21.6 ± 1.3	60.3 ± 1.8	75.7 ± 1.7	80.8 ± 1.6

(*) Mean ± standard error.

Comments

Dicker (1953), in rats anesthetized with ethanol found no antidiuretic activity in blood. Similar results were observed by Giere (1954), in decapitated rats, and by Birnie (1950), in hypophysectomized rats.

In the course of our experiments, some antidiuretic potency was always found. This effect cannot depend on pituitary secretion during bleeding, because the gland was isolated from the general circulation. Considering that other types of anesthesia produce a strong stimulation of the hypophysis (Dicker, 1953; Ginsburg, 1953; Giere, 1954) it would be possible that Avertin, the anesthesia used here, might have the same effect, as well. This would explain our positive results.

The greater antidiuretic activity obtained with the blood of adrenalectomized animals confirms the result reported by Birnie (1949).

References

(1) Ames, R. G., van Dyke, H. B.: *Endocrinology*, 1952, *50*, 350.
(2) Birnie, J. H., Jenkins, R., Eversole, W. J., Gaunt, R.: *Proc. Soc. exp. Biol.* N. Y., 1949, *70*, 83.
(3) Birnie, J. H., Eversole, W. J., Boss, W. R., Osborn, C. M., Gaunt, R.: *Endocrinology*, 1950, *47*, 1.
(4) Burn, J. H.: *Quart. J. Pharmacol.*, 1931, *4*, 517.
(5) Dicker, S. E., Ginsburg, M.: *Brit. J. Pharmacol.*, 1950, *5*, 497.
(6) Dicker, S. E.: *J. Physiol.* (Lond.), 1953, *122*, 149.
(7) Giere, F. A., Eversole, W. J.: *Science*, 1954, *120*, 395.
(8) Ginsburg, M., Heller, H.: *J. Endocr.*, 1953, *9*, 274.

MECHANISM OF THE EFFECT OF THYROXINE ON THE ADRENAL GLAND

CARMEN GRADO, EDITH DUBROCK, and JAIME TALESNIK

(Institute of Physiology and Department of Physiopathology, University of Chile, Medical School, Casilla 6510, Santiago de Chile)

IT has been shown that if thyroxine is given to adrenalectomized rats, a remarkable increase in the severity of adrenal insufficiency is provoked and premature death of the animals occur. Survival time depends on the dose of thyroxine used and varies between 3 to 8 days; calorigenesis is diminished as a consequence of adrenalectomy and the thyroxine administration is not followed by an increase in oxygen consumption.

The restitution of the metabolic response to thyroxine is obtained by substitution treatment with cortical extracts. Studying the "permissive" action of some corticosteroids (making use of Ingle's expression) ([18, 29]), it has been shown that very high doses of DCA are required by adrenalectomized animals to show a certain calorigenic response to thyroxine; on the other hand, with regard to the survival of these animals, the DCA showed rather poor effects ([4]). If cortisone is given instead, lengthening of the survival of adrenalectomized-thyroxinized rats is obtained. The increase of survival time keeps certain relation with the amount of cortisone used. Moreover, the increased oxygen consumption induced by thyroxine is similar in these cases to that obtained in normal conditions ([12, 14]).

The above mentioned experiments illustrate, once again, the importance of endocrine interrelations; in several instances the intimate relation of the thyroid activity with the functional state of the adrenal cortex has been emphasized ([3, 15, 16, 17, 20, 40]). Thus, while in hyperthyroidism the adrenal cortex is hypertrophic, it shows involution in hypothyroidism. The histological appearance shows that the adrenal alterations affects mainly the zona fasciculata ([44]). When thyroxine is given, a depletion in ascorbic acid (AA) and adrenal cholesterol

Received for publication, August 30th, 1959.

is obtained so it is suggestive that the morphological changes are accompanied by functional modifications (1).

In this paper, one of the possible mechanisms by which the thyroid hormone determines hypertrophy and probably, increase in the cortico-adrenal function was studied. Experiments were carried out on the effects of thyroxine in hypophysectomized rats; it was also intended to obtain information on the degree of cortical activity by measuring the level of AA in the adrenal gland. It is known that AA levels, as well as those of cholesterol, decrease when the adrenals are activated with ACTH (33, 34). Moreover, the depletion of AA (7, 19, 23, 25, 26, 32, 38) and cholesterol (10, 11, 21, 27, 41) is observed in normal rats that are subjected to stress conditions; there are arguments suggesting that these functional changes are induced through an antero-pituitary mechanism since they fail to be observed in hypophysectomized animals (5, 22, 28).

Methods

Albino rats were used, the animals were kept at controlled temperature of about 26° C and fed ad libitum.

The calorigenesis was followed by daily measurement of oxygen consumption in periods of 30 minutes using a closed circuit system (24).

The results expressed as normal metabolic rate (NMR) will be referred to the oxygen consumption per 100 g of body weight measured at 28° C without previous food deprivation.

Ascorbic acid determinations were made in:

A) Female rats weighing 110-115 g which were injected subcutaneously with:

1) 2 ‰, thyroxine (DL-thyroxine, British Drug Houses) in alcaline saline solution.

2) 2 ‰, cortisone acetate in saline solution (*).

B) Thyroidectomized female rats, with similar weight to the above mentioned. Thyroidectomy was performed under ether anaesthesia.

C) Hypophysectomized male rats, 120-125 g, treated in the same way as group A. Hypophysectomy (ether anaesthesia) was carried out according to Griffith and Farris's technique (9) with a slight modification, using tracheotomy with hypodermic needle Nº 16, which permits an easier operation, thus limiting danger of asphyxia or hemorrhage. Five per cent sugar was supplied with the water postoperatorily. Animals were allowed to recover for 48 hours before the injection of thyroxine or cortisone was started. The effectiveness of the hypophysectomy was controlled at the autopsy. The control animals were injected with equivalent volumes of saline solution as in groups injected with cortisone or thyroxine.

The animals were killed by a blow on the head and bled by the abdominal aorta, the adrenals removed, separately weighed and the ascorbic acid was measured according to Sayers & Sayers and adaptation (35) of the Roe and Kuether method (30). There was no significant difference between right and left glandular weights and their content in ascorbic acid, thus the results shown are the average value obtained from both glands.

(*) The authors are indebted to Dr. H. Molitor from the Merck Institute for Therapeutic Research, Rahway, New Jersey, U.S.A., for his generous supply of *Cortone* Merck.

For the statistical analysis of the results the standard error of the mean was calculated from

$$\sigma = \sqrt{\frac{\Sigma\, d^2}{n\ (n^{-1})}}$$

Results

Series I. Influence of thyroxine administration on the adrenal ascorbic acid content.

Fig. Nº 1 shows the metabolic rate response, weight changes and ascorbic acid of adrenal gland of animals killed at different intervals after daily treatment with 10 μg of thyroxine per gram of body weight.

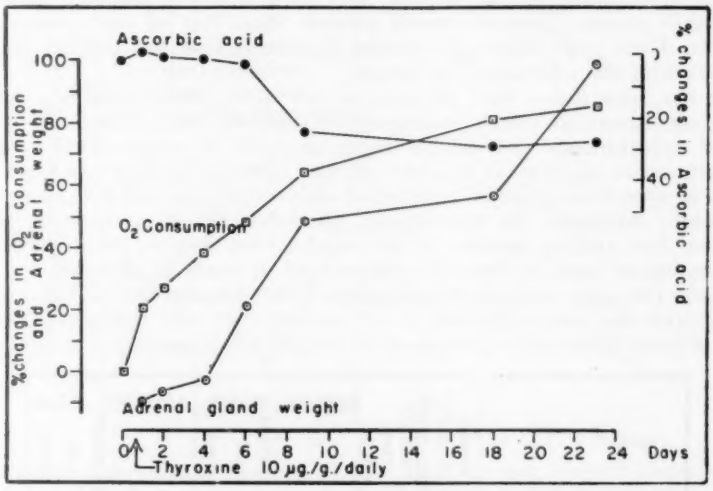


FIG. 1.—*Mean values of metabolic rate response and changes in adrenal gland weight and ascorbic acid content in rats treated with thyroxine.*

As it can be seen, the increased oxygen consumption starts about 24 hours after the beginning of the treatment, while the growth of the adrenal glands becomes evident only on the 5th or 6th day. On the other hand, the alterations in the ascorbic acid content are apparent only after 8 days, when the increased oxygen consumption reached about 65 % and the glands were nearly 50 % over the initial weight. Afterwards, oxygen consumption and glandular weight continued to increase progressively while the ascorbic acid maintained the level reached on the 9th day. As a side line observation it was seen that at the end of the long thyroxine treatment the adrenals showed spotted hemorrhages.

Thyroxine in doses of 10 μg/g body weight/day, was also given to 12 animals for 8 days in order to obtain more information about the time required to induce the fall of ascorbic acid. The ascorbic acid and glandular weight values ranged between the normals and the ones observed at the 9th day of treatment with thyroxine. The average ascorbic acid content was 380 $\pm$ 63 mg/100 g and the mean glandular weight 15.05 $\pm$ 4.0 mg/100 g.

The effects of thyroidectomy and thyroxine administration are shown in table 1. As said before, daily O_2 consumption determinations were carried out and it could be seen that animals injected with 15 μg/g/day raise their metabolism more rapidly than those receiving 10 or 5 μg/g/day thus, on the 7th day of treatment with 15 μg, the metabolism increased approximately 70 %, while the group treated with 5 μg/g/day took 12 days to reach a similar level. The decrease in ascorbic acid content of the adrenals reached similar values in the rats treated with 5, 10 or 15 μg/g/day of thyroxine. It is interesting to point out, nevertheless, that glandular hypertrophy is significantly greater in those animals receiving 5 μg per 12 days.

On the other hand, while thyroidectomized animals showed a tendency to diminish their oxygen consumption, the adrenal gland's weight kept within the normal range. However, the ascorbic acid content was significantly greater in the glands of the thyroidectomized animals than that of the corresponding controls. These experiments favour the hypothesis that the thyroid hormone interacts with the adrenocortical activity.

It has been shown that an extra supply of cortisone induces a decrease of the adreno-cortical activity; therefore investigations were carried out to see if cortical hyperfunction induced by thyroxine could be counteracted by means of simultaneous injections of cortisone. Results obtained are shown in table Nº 1. It can be seen that cortisone supplied in daily doses of 2 and 4 mg for 9 days determines involution in the adrenal glandular size in proportion to the cortisone dose and an increase in the ascorbic acid content, which appears to be independent from the dose of cortisone used. It could be observed that when thyroxine (10 μg/g/day) was given together with cortisone, the increased oxygen consumption was similar to that of rats treated only with thyroxine. The size of the adrenal gland shows persistence of atrophy while ascorbic acid diminishes.

TREATMENT	DAYS	% changes from normal body weight	% changes from N.M.R.	ADRENAL WEIGHT mg %	ADRENAL WEIGHT % changes from normal	ASCORBIC ACID mg %	ASCORBIC ACID % changes from normal
Normal				12·4±1·0	0	472·± 7	0
Thyroidectomized	21	+19	-.44	11 7±06	- 5·6	552 ±14	+ 17
Thyroxine 5 µg/g	12	- 4·8	+65	24·9±1·0	+100	361±22	- 23
Thyroxine 10µg/g	9	- 9·7	+64	19·2±06	+ 60	363± 7	- 23
Thyroxine 15µg/g	7	- 9·8	+70	16·7±08	+ 35	348± 8	- 26
Cortisone 2 mg	9	0	+ 84	10 6±06	- 14	528±22	+ 11·8
Cortisone 4 mg	9	- 6·4	+ 89	7·6±00	- 39	530± 11	+ 11·9
Thyroxine 10µg/g + Cortisone 2 mg	9	- 8	+55	11·9±1·0	- 11·3	411± 11	- 14·8
Thyroxine 10µg/g + Cortisone 4 mg	9	-10	+68	8·3±0·7	- 33	426± 16	- 9·8

TABLE 1.—*Effects of thyroidectomy and thyroxine on the weight and ascorbic acid content of adrenal gland. Each group consisted of 10 rats except the normal that had 5 at the 7th day; 6 for 9 days; 5 for 12 days and 4 for 21 days. Since no differences were observed among the latter, the results for the adrenal weight and ascorbic acid for the control group are the mean values of all normals.*

This is very evident if comparisons are done with values obtained from adrenal glands from rats treated only with cortisone; in fact, the level reached by the ascorbic acid content of the adrenals of rats treated with cortisone plus thyroxine was about 20 % below that of the above mentioned controls.

Series II. Influence of thyroxine in hypophysectomized rats.

In order to study the mechanism by which the thyroid acts on the adreno-cortical activity, thyroxine (10 μg/g/day) was given to hypophysectomized rats.

The body weight curve of hypophysectomized animals levels off while their oxygen consumption diminishes in about 30 %. It was also verified, once more, that adreno-cortical ascorbic acid content decreases as involution of the gland occurs after hypophysectomy; gross examination shows the adrenals of a pale color and the fatty tissue that normally surrounds the gland had disappeared.

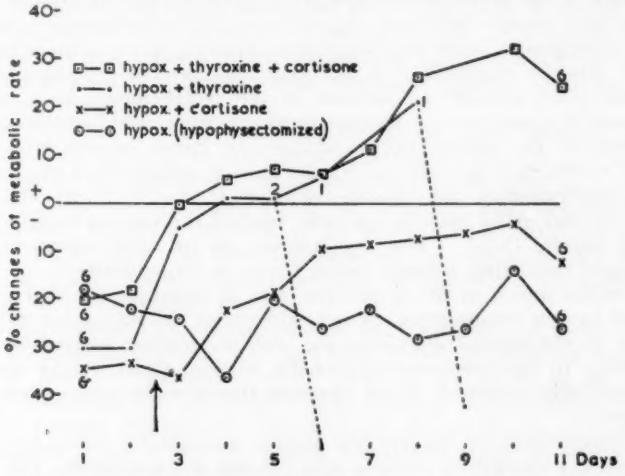


FIG. 2.—*Action of thyroxine in hypophysectomized rats. Thyroxine treatment started at the arrow. Numbers over the symbols refer to the number of animals studied. (Discussed in text.)*

After the eleventh day of hypophysectomy, the glandular weight as well as the ascorbic acid were stabilized, facts that agree with what other authors have observed ([6, 41]).

Hypophysectomized animals injected with 10 μg/g thyroxine daily, died between the 2nd and the 5th day after the treatment started. In Fig. 2 the percentage modification of oxygen consumption of hypophysectomized and thyroxinized-hypophysectomized rats are shown. It may be seen that under thyroxine the metabolic rate reaches the preoperatory level and in some cases higher values than the average normal metabolic level were obtained. In some cases it could be observed that some hours before death the oxygen consumption dropped to about 50 % of its original value. The dotted lines in the graph show the premortal lowering of metabolism.

Another group of hypophysectomized rats were injected simultaneously with thyroxine 10 μg/g body weight/day and cortisone 2 mg/rat/day. In these conditions the oxygen consumption rose quite similarly to that of normal animals treated with thyroxine. It should be emphasized the fact that hypophysec-

tomized rats treated with cortisone survive the action of thyroxine much longer than the animals that had no supplementary treatment with cortisone.

The third group plotted in the graph corresponds to hypophysectomized rats treated only with 2 mg of cortisone daily; it is interesting to note that the animals' lowered metabolic rate tends to approach to NML.

Discussion

As mentioned above the increase in oxygen consumption induced by thyroxine is not "permitted" when there is a shortage in adreno-cortical hormone; furthermore, the survival time of adrenalectomized animals is shortened by the thyroid hormone ([13]).

The lack of the adrenals can be replaced with cortical extracts or cortisone. It has also been shown that when experimental hyperthyroidism is in progress, a normal calorigenic answer and lengthened survival is obtained when adrenalectomized rats are supplied with adequate amounts of cortisone ([14]). These experiments point towards an increase of the requirements in corticosteroids in experimental thyrotoxicosis. Greater requirements would be satisfied through an increment of the adreno-cortical activity; in favour of this last conclusion is the well known fact that adrenal hypertrophy is produced in hyperthyroidism while in hypothyroidism the opposite occurs ([6, 14, 19, 20, 22, 40, 44]). On the other hand, the adrenal gland activity has been studied through its ascorbic acid and cholesterol content ([1, 43]). These last indexes are of value when accompanied by other signs indicating adrenal cortex hyper or hypoactivity.

The results shown in this paper are clear in pointing out that when there is increased oxygen consumption, giving evidence of the thyrotoxic state, there is an increase in the adrenal glandular size with diminished adrenal ascorbic acid concentration. In thyroidectomized rats the adrenal gland weight and ascorbic acid content were modified in an opposite direction in comparison with the former case.

The hypertrophy of the adrenal glands induced by thyroxine treatment starts before any changes in ascorbic acid content are noticeable. The reduction in ascorbic acid reaches a steady level though the gland hypertrophy follows a parallel increase with the oxygen consumption induced by the thyroxine treatment. Different explanations could be given for the apparent disagreement between glandular growth and ascorbic acid reduction. Cortex adaptation to the new condition of increased hormone production demands could be considered, as it has already been suggested ([31]). If one considers the possibility that the adrenal cortex activation by thyroxine is accomplished through the pituitary, the possibility exist that the mediators acting on the adrenals may act independently on the structure and on the hormone secretion ([18]).

When thyroxine was given to hypophysectomized rats a response was obtained with increased oxygen consumption in quite a similar way as normal ones do. The acceleration of the metabolic rate in this condition, that has also been observed by others ([8]), raises the problem of the differences obtained when thyroxine is given to adrenalectomized animals. The failure of adrenalectomized rats to intensify their metabolism when given thyroxine has been clearly shown to be due to lack of the "permissive" action of the corticosteroids.

The adrenal insufficiency induced in hypophysectomized animals is relatively balanced, as evidenced by the fact that survival is longer when compared with adrenalectomized animals. From this point of view, the main difference would

be then that the atrophy of the adrenal glands in hypophysectomy would leave enough gland tissue able to secrete reduced amounts of corticosteroids that are enough to keep these animals alive, as it has been shown for aldosterone and corticosterone in hypophysectomized rats ([36]). The balance can be broken when there is an increase in requirements of cortical hormones and the production capacity of the adrenal cortex is surpassed with development of adrenal insufficiency and consequent death. This point of view is supported by the experiments where prolonged survival was obtained when cortisone was given to hypophysectomized rats treated with thyroxine. As already mentioned ([12, 14]) a similar protection with cortisone was obtained when this substance was given to adrenalectomized rats.

No conclusions can be drawn from the ascorbic acid contents and glandular weight values found in hypophysectomized rats treated with thyroxine since these animals died before the 9th day of treatment; on the other hand, the adrenal cortical atrophy that follows hypophysectomy is accompanied by a drop in the ascorbic acid content and therefore this experiment does not admit the possibility of a clear cut interpretation.

When cortisone is administered to normal rats, inhibition of the adrenal gland is obtained as it can be seen by the increase in ascorbic acid content and decreased size of the gland; very similar results were obtained as a consequence of thyroidectomy. Simultaneous supply of cortisone and thyroxine produces adrenal atrophy accompanied however by a drop in ascorbic acid content of the gland. Thus, when the increased demands for costicosteroids induced by thyroxine, are filled by exogenous cortisone, it seems that the antero-pituitary slows down its corticotrophic action.

As already mentioned, the amount of cortisone given in these experiments could not prevent the ascorbic acid depletion when thyroxine was injected. The explanation for this phenomena could probably be found in the separation that seems to exist between citotrophic antero-pituitary action and the stimulating action on hormone production. The intricacy of the results could be due to the competitive action of the stimulating and depressor effects of thyroxine and cortisone respectively on the antero-pituitary ([31]).

We acknowledge the help of Dr. Hodgson in revising the manuscript.

SUMMARY

Some mechanisms involved in the modification of the adrenal activity due to lack or excess of thyroid hormone, were studied in the rat.

Thyroidectomy induces adrenal involution and an increase in the gland ascorbic acid concentration. On the other hand, thyroxine administration leads to adrenal hypertrophy and ascorbic acid depletion.

When thyroxine is given to hypophysectomized animals, the oxygen consumption increases as in normal conditions but the rat's survival is shorter. This latter effect can be counteracted by cortisone.

Relatively high doses of cortisone produce adrenal atrophy and increase ascorbic acid concentration. When cortisone is administered together with thyroxine the gross morphological adrenal changes induced by the latter are not present.

The results are discussed mainly from the viewpoint of endocrine interrelationships. Emphasis is placed on the adaptation of the adrenal cortex to conditions of increased corticosteroids requirement.

References

(1) Abelin, J.: *Helv. physiol. acta.*, 1943, *1*, C 81.
(2) Andrade, C. C.: Influencia de la corteza suprarrenal sobre la calorigénesis inducida por la tiroxina. *Tesis Inst. Fisiol.*, Univ. Chile, 1946.
(3) Baumann, E. J., Marine, D.: *Endocrinology*, 1945, *36*, 400.
(4) Cori, A., Cori, O., Talesnik, J.: *Personal Communication.*
(5) Covian, M. R.: *Rev. Soc. argent. Biol.*, 1949, *25*, 51.
(6) Çovian, M. R.: *Rev. Soc. argent. Biol.*, 1949, *25*, 26.
(7) De Majo, S.: *Rev. Soc. argent. Biol.*, 1947, *23*, 46.
(8) Eartly, H., Leblond, C. P.: *Endocrinology*, 1954, *54*, 249.
(9) Farris, E. J., Griffith, J. Q.: The rat in Laboratory Investigation, *2nd. ed. Philadelphia, J. B. Lippincott*, 1949.
(10) Gaunt, R., Cordsen, M., Liling, N.: *Endocrinology*, 1944, *35*, 105.
(11) Harkins, H. N., Long, C. N. H.: *Amer. J. Physiol.*, 1945, *144*, 661.
(12) Hoffmann, F.: *Comunicación Tercer Congreso Panamericano de Endocrinología, Santiago de Chile 1954.* Ed. Universitaria, 1956, *I*, 166.
(13) Hoffmann, F., Hoffmann, E. J., Talesnik, J.: *J. Physiol.*, (Lond.) 1948, *107*, 251.
(14) Hoffmann, F., Hoffmann, E. J., Knierim, F., Talesnik, J.: *Bol. Soc. Biol.* Stgo. Chile., 1952, *9*, 21.
(15) Koskins, R. G.: *J. Amer. med. Ass.*, 1910, *55*, 1724.
(16) Ingle, D. J., Higgins, G. M.: *Endocrinology*, 1938, *23*, 419.
(17) Ingle, D. J., Kendall, E. C.: *Amer. J. Physiol.*, 1938, *122*, 585.
(18) Ingle, D. J.:*J. Endocr.*, 1951-52, *8*, XXIII.
(19) Irwin, E., Buchanan, A. R., Longwell, B. B., Holtkamp, D. E., Hill, R. M.: *Endocrinology*, 1950, *46*, 526.
(20) Koelsche, G. A., Kendall, E. C.: *Amer. J. Physiol.*, 1935, *113*, 335.
(21) Levin, L.: *Endocrinology*, 1945, *37*, 34.
(22) Long, C. N. H.: *Rec. Prog. Hormone Res.*, 1948, *I*, 99.
(23) Ludewig, S., Chanutin, A.: *Endocrinology*, 1957, *41*, 135.
(24) Muñoz, O. E.: Acción de la tiroxina sobre la cuantía del metabolismo en ratas sometidas a dietas ricas en grasa y relativamente pobres en colina. *Tesis Inst. Fisiol.*, Univ. Chile, 1946.
(25) Nasmyth, P. A.: *J. Physiol.*, (Lond.) 1951, *112*, 215.
(26) Nasmyth, P. A.: *J. Physiol.* (Lond.) 1950, *110*, 294.
(27) Patt, H. M., Swift, M. N., Tyree, E. B., John, E. S.: *Amer. J. Physiol.*, 1947, *150*, 480.
(28) Pincus, G., Thimann, K. V., Thimann, K. V.: *The Hormones: Physiology Chemistry, and Applications, Academic Press*, New York, 1950, *II*, 152.
(29) Ralli, E. P.: Permissive action of adrenal cortical hormones. Adrenal Cortex. Transactions of the Fourth Conference 1952. *The Josiah Macy, Jr. Foundation*, 1953, 11.
(30) Roe, H. J., Kuether, A. C.: *J. biol. Chem.*, 1943, *147*, 399.
(31) Sayers, G.: *Physiol, Rev.*, 1950, *30*, 241.
(32) Sayers,G., Sayers, M. A., Tsan-Ying Liang, M. A., Long, C. N. H.: *Endocrinology*, 1945, *37*, 96.
(33) Sayers, G., Sayers, M. A., White, A., Long, C. N. H.: *Proc. Soc. exp. Biol.* N. Y., 1943, *52*, 200.
(34) Sayers, G., Sayers, M. A., Tsan-Ying Liang, M. A., Long, C. N. H.: *Endocrinology*, 1946, *38*, 1.
(35) Sayers, M. A., Sayers, G., Woodbury, L. A.: *Endocrinology*, 1948, *42*, 379.
(36) Singer, B., Stack-Dunne, M. P.: *J. Endocr.* 1955, *12*, 130.
(37) Stack-Dunne, M. P., Young, F. C.: *J. Endocr.*, 1951, *7*, LXVI.
(38) Skelton, F. R., Fortier, C., Selye, H.: *Proc. Soc. exp. Biol.* N. Y., 1949, *71*, 120.
(39) Steeples, G. L., Jensen, H.: *Amer. J. Physiol.*, 1949, *157*, 418.
(40) Tepperman, J., Engel, F. L., Long, C. N. H.: *Endocrinology*, 1943, *32*, 373.
(41) Tyslewitz, R.: *Endocrinology*, 1943, *32*, 103.
(42) Valenzuela, L. H.: Acción calorígena de extractos totales de corteza suprarrenal y acetato de desoxicorticosterona. *Tesis Inst. Fisiol.* Univ. Chile, 1947.
(43) Wallach, D. P., Reineke, E. P.: *Endocrinology*, 1949, *45*, 75.
(44) Wendler, H. D.: *Endocrinology*, 1947, *41*, 243.

INDICE

AUTORES

ACEVES, J., Méndez, C., Méndez, R.: Inhibición simpática periférica por los digitálicos con los nodos seno-auricular y aurículo-ventricular 143
ACEVES, J., Ver Méndez R. y Col. 158
ALAMILLA, M.: Ver Maldonado F. 157
ALANIS, J., López, E., Pulido, J.: La morfología del potencial H y algunos factores que la modifican 143
ALANIS, J.: Ver Rosenblueth A. y Col. 162
ALCARÁZ, M., Guzmán, C.: Modulación de los impulsos aferentes en el S. N. C. por la corteza cerebral y la formación recticular 144
ALCARÁZ, M.: Ver Guzmán, C. 153
ALCARÁZ, M.: Ver Harmony, T. y Col. 154
ALCOCER GUARON, C., Eckhaus Kraus, E., Villaseñor, C.: Efectos de la adrenalina y de la noradrenalina sobre la respuesta eléctrica del epitelio olfatorio de la rana 145
ALONSO DE FLORIDA, F., Cato, J., Blanco, L., Pardo, E. G.: Sobre la posible selectividad de algunos agentes bloqueadores para los ganglios automáticos parasimpáticos .. 146
ALONSO, F. E., Puebla, M. M., Fasciolo, J. C.: Indirect method for renin maesurement in the kidney 233
ALTIERI, M., Bazarque, P., Denti, A.: Preventive action of thyroidectomy with I^{131} on the diabetes of the rat 78
ALTIERI, N., Bazerque, P., Denti, A.: Curative action of yodothyroidectomy on the diabetes of the rat 228
ALTIERI, N., Bazerque, P., Denti, A.: Influence of the thyroid on the action of alloxan in the rat 229
ÁLVAREZ BUYLLA, R., Remolina, J.: The initiation of action potentials at pacinian corpuscles 178
ÁLVAREZ BUYLLA, R., Remolina, J., Deusth, E.: Estudio de la evolución ontogenética de algunos aspectos de la fisiología del miocardio 146
ANGUIANO LANDIN, G., Villasana, A., Montaño, C.: Adrenalina y adrenoxyl. Análisis del efecto inotrópico que inducen en el músculo esquelético 147
ANGUIANO, L. G.: Ver Villasana, A. 165
ANTUNES RODRÍGUEZ, J.: Influence of the cerebral cortex on the sexual cycle of the albino rat 230
APELBAUM, J.: Toxic and hypoglycemiant action of chlorpropamide 225
ARAMENDIA, P.: Ver Roncoroni, A. J. y Col. 55
ARAMENDIA, P.: Ver Taquini, A. C. y Col. 235
ASHKAR, E.: Ver Foglia, V. G. y Col. ... 226
ASTRADA, J. J., Caligaris, L. S. C. de: Detoxification of urine by dialysis in the determination of chorionic gonadotrophins 232
ÁVILA ROMO, E.: Ver Córdoba, F. y Col. 149
BADANO, B. N.: Ver Taquini, A. C. y Col. .. 235
BARNAFI, L., Vial, S., Croxatto, H.: The influence of the neck tourniquet on the blood antidiuretic potency of rats 338
BASILIO, C.: Ver Cabello, J. y Col. 286
BAZERQUE, P.: Ver Altieri, M. y Col., 78, 228 229
BEYER, C.: Cambios en la glucosa sanguínea por estimulación de la corteza cerebral 147
BINIA, A.: Ver Calvo, R. y Col. 234
BINIA, A.: Ver Zangheri, E. O. y Col. 232
BLANCO, L.: Ver Alonso de Florida, F. y Col. 146
BLAQUIER, J. A.: Action of different progestational steroides on the oviduct secretion of the toad *Bufo arenarum* Hensel 231
BRAVO, L. Ma.: Ver Villaseñor, G. y Col. .. 166
BRUST-CARMONA, H., Estrada-Robles, C.: Efectos de las lesiones cerebrales sobre una respuesta flexora condicionada 148
BRUST-CARMONA, H.: Ver Hernández-Peón, R. 154
BURGOS, M. H., Montes de Oca, H., Montorzi, N. M.: Fixation of Fe 59 by the "ductuli efferentes" of the hamster 231
BURGOS, M. H., Pisano, A.: Effect of purified gonadotrophins on the gonad of the *Bufo arenarum* Hensel after metamorphosis 76
BURGOS, M. H.: Ver Iriarte, J. V. 227
BURGOS, M. H.: Ver Yáñez, R. L. 230
CABELLO, J., Urbá Ruth, Prajoux Victoria, Basilio, C.: Induction of arginase synthesis in an auxotroph of Neurospora crassa 286
CALIGARIS, L. S. C. de: Ver Astrada, J. J. 232
CALVO, R., Chionetti, I. J., Fasciolo, J. C., Zangheri, E. O., Binia, A.: Mechanism of the pressor effect of the spider "Latrodectus mactans" venom. II) Study with Starling cardiopulmonary preparation 234
CALVO, R.: Ver Halvorsen, K. A. y Col. .. 77
CARDEZA, A. F.: Ver Lacy, P. E. 77
CARDEZA, A. F.: Ver Lacy, P. E. y Col. .. 230
CARDEZA, A. F.: Ver Penhos, J. C. 231
CARLINI, E. A., Sampaio, A. H., Paiva, A. C. M.: Vascular reactivity of rats with desoxycorticosterone and metacorticoid hypertension 138
CASTRO, E.: Ver Guzmán, J. y Col. 152
CATO, J.: Ver Alonso de Florida, F. y Col. .. 146
CATO, J.: Ver Vázquez, F. y Col. 164
CEREIJIDO, M.: Experimental modifications in the intrarenal pressure of dogs and rats 75
CÍCERO, R., Méndez, C., Méndez, R.: La recuperación del corazón después del paro cardíaco inducido por potasio 149
CÓRDOBA, F., Meza Gómez Palacio, M., Ávila Romo, E.: Bioquímica de la exfoliación vaginal. I. - Aminoácidos celulares y proteínas del fuído 149
CORRADO, A. P., Ramos, A. O., Rocha e Silva, M.: On possible central effects of bradykinin in the cat 222
CORRADO, A. P.: Ver Rocha e Silva, M. y Col. 223
CORREA DURÁN, A.: Ver Duomarco, J. L. y Col. 267
COVIÁN, M. R., Migliorini, R. H., Tramezzani, J. H.: Endocrine changes in hemidecorticate rats 24
COVIÁN, M. R.: Ver Migliorini, R. H. 226
CROXATTO, H.: Ver Barnafi, L. y Col. 338
CROXATO, H.: Ver Labarca, E. 123
CHIONETTI, J. I.: Ver Calvo, R. y Col. 234
CHIONETTI, J. I.: Ver Zangheri, E. O. y Col. 232
DE CORRAL, J. M.: Action of steroids on the ovulation of the toad *Bufo arenarum* Hensel 77
DE LA RIVA, I.: Ver Taquini, A. C. y Col. 235
DENTI, A.: Ver Altieri, M. y Col., 78, 228. 229
DEUSTH, E.: Ver Álvarez Buylla, R. y Col. 146
DOHI, S. R.: Ver Valeri, V. y Col. 223
DOUGLAS, R.: Ver Van Den Beld, C. y Col. 273
DUBROCK, EDITH: Ver Grado Carmen y Col. 341
DUOMARCO, J. L., Giambruno, C. E., Correa Durán, A.: The pressure in the different zones of the pericardium 267

EBERSTADT, P.: Ver Erlij, D. y Col. 150
EBERSTADT, P.: Ver Rudomín, Z. P. y Col. 209
ECKHAUS KRAUS, E.: Ver Alcocer Guarón, C. y Col. 145
ERLIJ, C., Rudomín, P., Eberstadt, P.: Influencia de la hipoxia e hipoventilación sobre la suma temporal y espacial del centro cardioinhibidor 150
ERLIJ, D.: Ver González Serratos, H. 152
ERLIJ, D.: Ver Rudomín, Z. P. y Col. 209
ESTRADA-ROBLES, C.: Ver Brust-Carmona, H. 148
FASCIOLO, J. C., Halvorsen, K., Zangheri, E., Fernández, F. O.: Comparison between bradykinin and substance 226
FASCIOLO, J. C.: Ver Alonso, F. E. y Col. 233
FASCIOLO, J. C.: Ver Calvo, R. y Col. 234
FASCIOLO, J. V.: Ver Halvorsen, K. A. y Col. 77
FASCIOLO, J. C.: Ver Zangheri, E. O. y Col. 232
FELS, E.: Inactivation of oestrogenic hormone and experimental tumorigenesis of the ovary 227
FELS, E.: Intrasplenic ovary graft in rats with ligature of the testicular pedicule 232
FERMOSO, J. D.: Ver Taquini, A. C. y Col. .. 235
FERNÁNDEZ, A., Roldán, E.: Efecto de la atropina sobre el sistema nervioso central .. 151
FERNÁNDEZ, F. O.: Ver Fasciolo, J. C. y Col. 226
FERNÁNDEZ, J.: Ver Monge, C. y Col. 50
FOGLIA, V. G., Torres, H. N., Moguilevsky, J. A., Ashkar, E.: Action of D. D. D. on the content of adrenal ascorbic acid in different animal 226
GARCÍA RAMOS, J.: Comparación entre las adaptaciones circulatorias y respiratorias a la hipoxia y al ejercicio físico 151
GARCÍA RAMOS, J.: On the integration of respiratory movements 246
GARCÍA RAMOS, J., López Mendoza, E.: On the integration of respiratory movements. II. The integration at spinal level 257
GARCÍA RAMOS, J., Reynaud, A. R.: Circulatory and respiratory adjustments to hipoxia and to light exercise in normal young men 169
GARRIDO, C. M.: Resistance to cold of adrenalectomized rats 232
GARRIDO, C. M.: Action of different substances on the resistance to cold of normal and adrenalectomized albino rats 233
GIAMBRUNO, C. E.: Ver Duomarco, J. L. y Col. 267
GÓMEZ, A.: Ver Olivera, H. y Col. 159
GONZÁLEZ, R.: Ver Roncoroni, A. J. y Col. 55
GONZÁLEZ SERRATOS, H., Erlij, J. D.: Algunas características del reflejo depresor coronario 152
GRADO, CARMEN, Dubrock, Edith, Talesnik, J.: Mechanism of the effect of thyroxine on the adrenal gland. 341
GUTIÉRREZ, A.: Ver Moguilevsky, J. A. y Col. 234
GUZMAN, C., Alcaráz, M.: Variaciones correlativas de los potenciales evocados por estímulos indiferentes y significativos 153
GUZMAN, C.: Ver Alcaráz, M. 144
GUZMAN, C.: Ver Harmony T. y Col. 154
GUZMAN, J., Olivera, H., Castro, E., Laguna, J.: Efectos de esteroides en el embrión del pollo: Acción de derivados fluorados sobre el metabolismo inorgánico y de los carbohidratos 152
GUZMAN, J.: Ver Laguna, J. y Col. 155
GUZMAN, J.: Ver Olivera, H. y Col. 159
GUZMAN, J.: Ver Villaseñor, G. y Col. 166
HACHEN CARMEN DEL CAMPO DE: Adrenocortical hormone in human blood 69
HALVORSEN, K. A., Fasciolo, J. C., Calvo, R., Chionetti, I.: A vasodilator substance present in purified angiotensin preparations 76
HALVORSEN, K. A., Fasciolo, J. C., Calvo, R.: Pressor and vasoconstrictor activity of angiotensin I compared with angiotensin II 77
HALVORSEN, K.: Ver Fasciolo, J. C. y Col. 226
HARMONY, T., Alcaráz, M., Guzmán, C.: Modificaciones de la actividad funcional del área motora a través de los sistemas aferentes 154
HEINRICH, J. J.: Ver Mancini, R. E. y Col. 229
HERNÁNDEZ-PEÓN, R., Brust-Carmona, H.: Influencias descendentes sobre la transmisión aferente de impulsos táctiles a nivel de la médula espinal en gatos despiertos 154
HORNA EDDA: Ver Monge, C. y Col. 50
HOUSSAY, A. B., Nallar, R., Saurer, Eva I.: Action of testosterone propionate upon the growth of hair in rats 35
HOUSSAY, A. B., Nallar, R., Saurer, Eva I.: Action of testosterone propionate on the hair growth of the white rat 76
Illanes, A.: Ver Penna, M. y Col. 66
IRIARTE, J. V., Burgos, M. H.: Inhibition of the spermiating effect of chorionic ganadotrophin 227
IZQUIERDO, I.: Ver Mancini, R. E. y Col. 229
KRAEMER, A.: Ver Osorio, J. A. 128
KRETSCHMER, R.: Ver Olivera, H. y Col. 159
LABARCA, E., Croxatto, H.: Effect of oxytocin and of acetazoleamide on the urinary excretion of water, sodium and pottasium in the rat 123
LACY, P. E., Cardeza, A. F.: Electron microscopy of pancreas, liver and salivary glands in guinea pigs treated with cobaltous chloride 77
LACY, P. E., Cardeza, A. F., Wilson, W. D.: Electron microscopy of the pancreas in rats treated with glucagon 230
LAGUNA, J., Olivera, H., Piña, E., Riviello, H., Guzmán, J.: Efectos de esteroides en el embrión de pollo 155
LAGUNA, J.: Ver Guzmán, J. y Col. 152
LAGUNA, J.: Ver Olivera, H. y Col. 159
LAGUNA, J.: Ver Villaseñor, G. y Col. 166
LANDA, A.: Ver Ondarza, R. y Col. 160
LAVIERI, J. C.: Ver Mancini, R. E. y Col... 288
LEWIS, J. T.: Braun Menéndez, E. (nota necrológica) 1
LICO, M. C.: Method of permanent lateral cannulation in the aorta of the toad 75
LÓPEZ, E.: Ver Alanís, J. y Col. 143
LÓPEZ, E.: Ver Rosenblueth, A. y Col. 162
LÓPEZ MENDOZA, E.: Ver García Ramos .. 247
LOUBATIERES, A.: I. - Etude experimentale des arylsulfonamides hypoglycemiants. II. - Mecanisme d'action des arylsulfonamides hypoglycemiants chez l'homme normal ou diabetique 79
LLAMAS, R., RolónArias, Ma. D.: Efecto de la ametopterina (ácido amino-4-metil-N-10-pteroilglutámico) sobre los ácidos ribonucleico y desoxiribonucleico de las fracciones celulares del hígado del ratón 156
MAGAÑ, J. I., Pardo, E. G.: Influencia de diversas aminas simpaticomiméticas sobre las respuestas vasculares a la epinefrina y al L-arterenol 157
MAGAÑA, J. L.: Ver Pardo, E. G. 161
MALDONADO, F., Alamilla, M.: Efecto del clorhidrato de meta-oxifenil-amino-etanol sobre la recuperación del débito cardíaco después de paro provocado 157
MALINOW, M. R.: The effects of estrogens on experimental atherosclerosis 313
MALINOW, M. R.: Ver Moguilevsky, J. A. y Col. 234
MANCINI, R. E., Narbaitz, R., Lavieri, J. C.: Origin and development of germinal epithelium and Sertoli cells in the human testicle. Cytological, cytochemical and quantitative study 228
MANCINI, R. E., Penhos, J. C., Izquierdo, I., Heinrich, J. J.: Effects of repeated insulin coma on the testicle of the rat 229
MARDONEZ, J.: Ver Penna, M. y Col. 66
MASSIEU, G., Ortega, G. B.: Nuevos datos sobre la acción hiperglucemiante de la 3-acetilpiridina 158
MÉNDEZ, R.: Ver Aceves, J. y Col. 143
MÉNDEZ, R.: Ver Cicero, R. y Col. 149
MÉNDEZ, C.: Ver Méndez, R. y Col. 158
MÉNDEZ, R., Méndez, C., Aceves, J.: Acción de los glucósidos cardíacos sobre el efecto de los estímulos adrenérgicos en el período refractario de la transmisión auriculo-ventricular 158
MEZA GÓMEZ PALACIO, M.: Ver Córdoba, F. y Col. 149

MIGLIORINI, R. H., Covián, M. R.: Seminal vesicles and ovaries in hemidecorticated mice 226
MIGLIORINI, R. H.: Ver Covián, M. R. y Col. 24
MIGLIORINI, R. H.: Ver Sesso, A. 5
MOGUILEVSKY, J. C., Gutiérrez, A., Malinow, M. R.: Effect of oestradiol on the male rat. Influence of way of administration .. 234
MOGUILEVSKY, J. C.: Ver Foglia, V. G. y Col. 226
MONGE, C., Ramírez, M., Fernández, J., Horna Edda: Relationship between serum creatinine, endogenous creatinine clearance and urinary creatinine 50
MONTAÑO, C.: Ver Anguiano Landín, G. y Col. 147
MONTES DE OCA, H.: Ver Burgos, M. H. y Col. 231
MONTFORT, E. I.: Ver Pérez Tamayo, R. y Col. 161
MONTORZI, N. M.: Ver Burgos, M. H. y Col. 231
MORA, J.: Ver Pérez Tamayo, R. y Col. .. 161
NALLAR, R.: Ver Houssay, A. B. y Col., 35, 76
NARBAITZ, R.: Ver Mancini, R. E. y Col. .. 228
NAVA RIVERA, A.: Acción del alcohol n-butílico sobre la prueba de tolerancia a la heparina 159
NEGRETE, J.: Ver Solís Cámara, P. 164
NEGRETE, J.: Ver Yankelevich Guillermina 167
OLIVERA, H., Guzmán, J., Kretschmer, R., Gómez, A., Laguna, J.: Estudios sobre regeneración hepática: efecto del alcohol y la colina dietéticos sobre la proteína, la grasa y algunas enzimas hepáticas 159
OLIVERA, H.: Ver Guzmán, J. y Col. 152
OLIVERA, H.: Ver Laguna, J. y Col. 155
OLIVERIO, L. G.: Ver Sesso, A. 13
ONDARZA, R., Sterling, L., Landa, A.: Nucleótidos libres en sangre leucémica 160
ORTEGA, G. B.: Ver Massieu,G. 158
OSORIO, J. A.: Kraemer, A.: Influencia da atrofia do cortice suprarrenal produzida pelo rhothance (DDD), sobre a glandula submaxilar do cao 128
PAIVA, A. C. M.: Ver Carlini, E. A. y Col. 138
PARDO, E. G., Magaña, J. L.: Contribución al estudio del fenómeno de taquifilaxia 161
PARDO, E. G.: Ver Alonso de Florida, F. y Col. 146
PARDO, E. G.: Ver Magaña, J. I. 157
PARDO, E. G.: Ver Vázquez, F. y Col. 164
PENHOS, J. C.: Changes produced by insulin on the effect of cortisone in the rat 228
PENHOS, J. C.: Interaction of Levo-thyroxine and folic acid or aminopterin in the rat .. 230
PENHOS, J. C.: Action of cortisone and sexual hormones in the rat 233
PENHOS, J. C., Cardeza, A. F.: Associate effect of levothyroxine and insulin in the rat .. 231
PENHOS, J. C.: Ver Mancini, R. E. y Col. .. 229
PENNA, M., Illanes, A., Ruíz, J., Mardones, J.: Effect of butyrate on ventricular fibrillation induced by lack of metabolites on the isolated guinea pig heart 66
PÉREZ TAMAYO, R., Mora, J., Montfort, E. I.: La naturaleza humoral de hiperesplenismo. Estudio experimental 161
PIÑA, E.: Ver Laguna, J. y Col. 155
PISANO, A., Pizarro, N.: Observations on the gonad's growth of *Bufo arenarum* Hensel 76
PISANO, A.: Ver Burgos, M. H. 76
PISANTY, J., Toscano, S.: Tratamiento de shock con corticosterona 162
PIZARRO, N.: Ver Pisano, A. 76
PROJOUX VICTORIA: Ver Cabello, J. y Col. 286
PUEBLA, M. M.: Ver Alonso, F. E. y Col. .. 233
PULIDO, J.: Ver Alanís, J. y Col. 143
RAMÍREZ, M.: Ver Monge, C. y Col. 50
RAMOS, A. O.: Ver Corrado, A. P. y Col. .. 222
RAMOS, A. O.: Ver Rocha e Silva, M. y Col. 223
REMOLINA, J.: Ver Álvarez Buylla, R. y Col. 146
REMOLINA, J.: Ver Álvarez Buylla, R. 178
REYNAUD, R.: Ver García Ramos, J. 169
RIVIELLO, H.: Ver Laguna, J. y Col. 155
ROCHA E SILVA, M., Corrado, A. P., Ramos, A. O.: Influence of sympatholytic agents and of reserpine on the hypotensive effect produced by bradykinin in cats 223
ROCHA E SILVA, M.: Ver Corrado, A. P. y Col. 222
RODRÍGUEZ DE SAMPAIO, J.: Ver Valeri, V. y Col. 223
ROLDÁN, E.: Ver Fernández, A. 151
ROLÓN ARIAS, Ma. D.: Ver Llamas, R. 156
RONCORONI, A. J., Aramendia, P., González, R., Taquini, A. C.: "Central" Blood volume in exercise in normal subjects 55
ROSENBLUETH, A., Alanís, J., López, E., Rubio, R.: La ley de Starling 162
RUBIO, R.: La influencia de la tensión sobre el mecanismo contractil durante el reposo y la contractura 163
RUBIO, R.: Las contradicciones isotónicas de los músculos estriados 298
RUBIO, R.: La distribución de la tensión en el músculo durante el reposo y la contracción 306
RUBIO, R.: Ver Rosenblueth, A. y Col. 162
RUBIO, R.: Ver Rudomín, Z. P. 163
RUBIO, R.: Ver Rudomín, Z. P. 194
RUDOMÍN, Z. P., Erlij, D., Eberstadt, P.: Influencia de la hipoxia e hipoventilación sobre la sumación temporal y espacial del centro cardioinhibidor 209
RUDOMÍN, Z. P., Rubio, R.: Actividad subliminal de los quimiorreceptores pulmonares 163
RUDOMÍN, Z. P., Rubio, R.: Actividad subliminal de los quimiorreflejos originados por el cianuro de potasio 194
RUDOMÍN, P.: Ver Erlij, D. y Col. 150
RUIZ, J.: Ver Penna, M. y Col. 66
RUSSEK, M.: On the mechanism of "external inhibition' 133
RUSSEK, M.: The action of weak histotoxic hypoxia on isolated nerve, neuromuscular preparation and sympathetic ganglion nictitating membrane preparation 188
SAMPAIO, A. H.: Ver Carlini, E. A. y Col. 138
SAURER, EVA I.: Ver Houssay, A. B. y Col., 35 76
SEGURA, E. T.: Effect of calcium mesooxalate on meta alloxan diabetes of rabbits 227
SESSO, A., Migliorini, R.: Nucleic acid content and amylase activity in the pancreas of the rat following adrenalectomy and cortisone administration 5
SESSO, A., Olivério, L. G.: The anabolic acction of cortisoneupon the exocrine pancreas of the suckling rat 13
SOLÍS-CÁMARA, P., Negrete, J.: Resistencia por tuberculencia al flujo intermiante 164
STERLING, L.: Ver Ondarza, R. y Col. 160
TALESNIK, J.: Ver Grado Carmen y Col. .. 341
TALESNIK, J. Ver Van Den Beld, C. y Col. 273
TAQUINI, A. C., Fermoso, J. D., Aramendía, P., De la Riva, I., Badano, B. N., Villamil, M. F.: Influence, of postural changes on ammonia excretion by the kidney in ammonium chloride loading 235
TAQUINI, A. C.: Ver Roncoroni, A. J. y Col. 55
TELLEZ CIRÓN, E.: Ver Villareal, R. 166
TORRES, H. N.: Ver Foglia, V. G. y Col. .. 226
TOSCANO, S.: Ver Pisanty, J. 162
TRAMEZZANI, J. H.: Ver Covián, M. R. y Col. 24
URANGA, JULIA V.: Intestinal water absorption in the toad 75
URANGA, JULIA V.: Water absorption by the toad's skin 225
URANGA, JULIA V.: Body weight increase in toads injected with vasopressin or toad's neurointermediate lobe 227
URANGA, JULIA V.: Influence of neurohypophyseal hormones and of hypophysis removal on the diuresis of the toad 228
URBA RUTH: Ver Cabello, J. y Col. 286
VALERI, V., Dohi, S. R., Rodríguez de Sampaio, J.: Exchanges in parabiotic rats studied in normal and nephrectomized animals 223
VAN DEN BELD, H., Douglas, R., Talesnik, J.: Functional modifications of the pacemaker and conducting systems in experimental hyper and hypothyroidism 273
VÁZQUEZ, F., Cato, J., Pardo, E. G.: Sobre las respuestas presoras al tetraetilamonio .. 164
VIAL, S.: Ver Barnafi, L. y Col. 338

VIDRIO, H.: Valoración semicontinua de la secreción gástrica y del efecto antisecretor de algunas drogas 165
VILLAMIL, M. F.: Ver Taquini, A. C. y Col. 235
VILLARREAL, R., Téllez Girón, E.: Secreción de ácido clorhídrico y actividad de sistemas enzimáticos oxidativos en mucosa gástrica .. 166
VILLASANA, A., Anguiano, G.: Efectos de la 5-hidroxitriptamina sobre el músculo esquelético de mamífero 165
VILLASANA, A.: Ver Anguiano Landín, G. y Col. 147
VILLASEÑOR, C.: Ver Alcocer Guarón, C. y Col. 145
VILLASEÑOR, G., Bravo, L. Ma., Guzmán, J., Laguna, J.: Grasa y coenzima "A" en los hígados de embriones de pollo tratados con cortisona 166
WILSON, W. D.: Ver Lacy, P. E. y Col. .. 230
YÁÑEZ, R. L., Burgos, M. H.: Autoradiographic study of I^{131} uptake by the thyroid in the toad 230
YANKELEVICH, GUILLERMINA, Negrete, J.: Los potenciales eléctricos en la zona de la placa motora durante la fatiga de transmisión neuromuscular en músculos curarizados .. 167
ZANGHERI, E. O., Binia, A., Fasciolo, J. C., Chionetti, J. I.: Comparative estudy of the adrenolytic action between total extract of "Rauwolfia serpentina" and "Rauwolfia schueli" 232
ZANGHERI, E. O.: Ver Calvo, R. y Col. .. 234
ZANGHERI, E.: Ver Fasciolo, J. C. y Col. .. 226

MATERIAS

Absorption (see intestinal) 75
Acetazoleamide (see Ocytocin) 123
3-acetilpiridina 158
Ácido clorhídrico 166
Adaptaciones circulatorias y respiratorias 151
Adrenal (see DDD) 226
Adrenal (see Thyroxine) 341
Adrenalectomized (see cold) 233
Adrenalina y Adrenoxyl 147
Adrenocortical hormones 69
Adrenolytic (see Rauwolfia) 232
Alcohol n-butílico 159
Alcohol (ver regeneración hepática) 159
Alloxan (see mesooxalate) 227
Alloxan (see thyroid) 229
Ametropterina 156
Aminopterin (see thyroxin) 230
Ammonia excretion 235
Amylase (see nucleic acid) 5
Angiotensin I and II 77
Antidiuretic 338
Área motora 154
Arginase 286
Aorta cannulation in toad 75
Atherosclerosis (see estrogens) 313
Atropina 151
Blood (see antidiuretic) 338
Blood volume 55
Bloqueadores 146
Bradykinin, 222, 223 226
Braun Menéndez, E. (Nota necrológica) 1
Butyrate 66
Cardioinhibidor (ver hipoxia) 209
Cerebral Cortex 230
Cerebrales lesiones 148
Cianuro (ver quimiorreflejos) 194
Cobalt (see pancreas) 77
Coenzima "A" (ver cortisona) 166
Cold, 232 233
Colina (ver regeneración hepática) 159
Contractura (ver tensión) 163
Corazón (ver glucósidos cardíacos) 158
Corazón (ver Hipoxia e hiperventilación) 150
Corazón (ver metaoxifenil-amino-etanol) 157
Corazón (ver Ontogenia) 146
Corazón (ver potasio) 149
Coronario (ver reflejo depresor) 152
Corteza cerebral, 144 147
Corticosterona 162
Cortisona, 13, 166, 228 233
Creatinine 50
Curare (ver placa motriz) 167
Chlorpropamide 225
Chorionic (see gonadotrophin), 227 232
DDD, 128 226
Desoxycorticosterone (see vascular reactivity) 138
Diabetes 228
Diabetes (see mesooxalate) 227
Diabetes (see thyroidectomy) 78
Digitálicos 143
Diuresis (see neurohypophysis) 228
Ductuli efferentes 231
Ejercicio (ver adaptaciones circulatorias y respiratorias) 151
Ejercicio (ver músculo) 306
Embrión de pollo 152
Endocrina in hemidecorticate 24
Esteroides, 152 155
Estómago (ver ácido clorhídrico) 166
Estómago (ver secreción gástrica) 165
Estrogens 313
Exercise (see Hypoxia) 169
Fibrillation (see butyrate) 66
Folic acid (see thyroxin) 230
Glucagon (see Pancreas) 230
Glucemia (ver corteza cerebral) 147
Glucósidos cardíacos 158
Gonadotrophin 227
Gonadotrophins 76
Gonadotrophin chorionic 232
Gonadal growth 76
Hair (see Testosterone), 35 76
Hamster (see ductuli) 231
Hemidecortication 226
Hemidecortication (see Cerebral cortex) 230
Hemidecortication (see endocrine chonges) .. 24
Heparina (ver alcohol n-butílico) 159
5-hidroxitriptamina 165
Hígado (ver cortisona) 166
Hígado (ver regeneración hepática) 159
Hiperesplenismo 161
Hiperglucemia (ver 3-acetilpiridina) 158
Hipoxia (ver adaptaciones circulatorias y respiratorias) 151
Hipoxia e hipoventilación, 150 209
Hypertension (see vascular reactivity) 138
Hypoglycemia (see chlorpropamide) 225
Hypoglycemia (see sulfonamides) 79
Hypoxia, 169 188
Insulin 228
Insulin coma 229
Insulin (see thyroxine) 231
Iodine (see thyroid) 230
Intestinal water absorption 75
Intrarenal Pressure 75
Kidney (see ammonia excretion) 235
Kidney (see Renin) 233
Latrodectus mactans venom 234
Leucemia (ver nucleótidos) 160
Ley de Starling 162
Mesooxalate 227
Metacorticoid hypertension (see vascular reactivity) 138
Metaoxigenil-aminoetanol 157
Modulación de los impulsos aferente 144
Músculo, 298 306
Músculo (ver 5-hidroxitriptamina) 165
Nephrectomized (see parabiosis) 223
Nerve (see hypoxia) 188
Neurohypophysis 228
Neurohypophysis (see vasopressin) 227
Neurohypophysis (see water absorption) 225
Neurospora (see arginase) 286
Noradrenalina (ver simpaticomiméticas) 157
Nucleic acid and amylase 5
Nucleicos ácidos (ver ametopterina) 156
Nucleótidos 160
Oestradiol 234
Oestrogenic hormone 227
Olfatorio 145
Ontogenia de la fisiología del miocardio 146
Ovary graft 232
Ovary (see hemidecortication) 226
Ovary (see tumorigenesis) 227
Oviduct secretion 231
Ovulation 77
Oxytocin and acetazoleamide 123
Pacinian corpuscles 178

Páncreas, 77 230
Páncreas (see cortisone) 13
Páncreas (see nucleic acid) 5
Parabiosis 223
Pericardium 267
Placa motriz 167
Potasio 149
Potencial H 143
Potenciales evocador 153
Proceedings Argent. Soc. Biol., 75 225
Proceedings Soc. Biol. Ribeirao Preto (Brasil) 222
Quimiorreceptores pulmonares 163
Quimiorreflejos 194
Rauwolfia serpentina and schueli 232
Reflejo depresor coronario 152
Regeneración hepática 159
Renin measurement 233
Reserpine 223
Resistencia por turbulencia 164
Respiratory movements, 246 247
Reticular 144
Salivary glands (see pancreas) 77
Secreción gástrica 165
Segundo Congreso de la Sociedad Mexicana de Ciencias Fisiológicas (Proceedings) 143
Seminal vesicle (see hemidecortication) 226
Sexual hormones (see cortisone) 233
Shock (see corticosterona) 162
Simpaticomiméticas 157
Sistema nervioso central 151
Skin (see water absorption) 225
Spermiation (see gonadotrophin) 227
Starling, ley de 162
Steroid (see ovulation) 77
Submaxilar 128
Substance V (see bradykinin) 226
Sulfonamides 79
Suprarrenal 128
Sympathicolytic 223
Táctiles, impulsos 154
Taquifilaxia 161
Tensión 163
Testicle 228
Testicle (see Insulin) 229
Testosterone, 35 76
Tetraetilamonio 164
Thyroid, 229, 230 273
Thyroidectomy, 78 228
Thyroxin, 230, 231 341
Tumorigenesis (see oestrogenic hormones) 227
Turbulencia (ver resistencia) 164
Vagina 149
Vascular reactivity 138
Vasodilator substance 76
Vasopressin 227
V substance (see vasodilator substance) 76
Water absorption 225

COMPAÑÍA IMPRESORA ARGENTINA S. A.
Alsina 2049 – Buenos Aires

REGLAMENTO DE PUBLICACION

Acta Physiologica Latinoamericana publica artículos originales inéditos o que hayan sido publicados previamente en forma parcial o completa en alguna revista local.

Los trabajos deben ser enviados al Jefe de Redacción del país de origen. Si no existiera Comité Editorial, deberán ser enviados a la Secretaría de Redacción de Acta Physiologica Latinoamericana, Obligado 2490, Buenos Aires, Argentina. La revista no se responsabiliza por los daños sufridos por el manuscrito o por su pérdida. Se recomienda a los autores conservar una copia completa de los trabajos que envíen por correo.

Preparación de los trabajos: Deberán ser escritos a máquina en papel tamaño carta, no transparente, a doble espacio y con amplio margen. Las ilustraciones deberán estar numeradas (fig. 1, fig. 2, etcétera), y llevar al pie una leyenda clara y concisa. Las fotografías hechas en papel brillante, nítidas. Los gráficos y diagramas, dibujados con tinta china sobre fondo blanco, listos para reproducir.

Se publicarán trabajos escritos en castellano, portugués, francés o inglés. Los que estén escritos en castellano o portugués deberán contener al final un resumen en inglés.

Las citas bibliográficas se harán en el texto mediante números [por ej.: algunos autores [1, 2]) y en especial Jones [3]] o autores y año [por ej.: (Breslauer, 1919)]. Al final del trabajo la bibliografía se ordenará alfabéticamente y con numeración progresiva, en el primer supuesto, y alfabéticamente en el segundo. Para las abreviaturas de las revistas, etc., se seguirán las recomendaciones del World List of Scientific Periodicals. La disposición de tales citas debe ajustarse a los ejemplos siguientes:

(1) HOUSSAY, B. A., LEWIS, J. T., ORÍAS, O., BRAUN MENÉNDEZ, E., HUG, E., FOGLIA, V. G., LELOIR, L. F.: *Fisiología Humana*, 3ª edición, El Ateneo, Buenos Aires 1954.

(2) WHITTEMBURY, G., RAMÍREZ, M., FERNÁNDEZ, J., MONGE, C.: *Acta physiol. latinoamer.*, 1955, *5*, 117.

De acuerdo con el carácter del artículo (artículo de conjunto o comunicación original) constará o no el título completo de los trabajos citados en la bibliografía.

Las medidas y símbolos deben expresarse de acuerdo con las recomendaciones de la Comisión de Símbolos, Unidades y Nomenclatura de la Unión Internacional de Física, aprobados en Amsterdam, en junio de 1948 (*Cienc. e Invest.*, 1949, *5*, 433).

SE EXPONEN A CONTINUACION ALGUNAS ABREVIATURAS COMUNES

	Castellano	*Inglés*		*Castellano*	*Inglés*
metro	m	m	milisegundo	ms	msec
centímetro	cm	cm	litro	l	l
milímetro	mm	mm	centímetro cúbico	cm^3	cc
micrón	μ	μ	mililitro	ml	ml
milimicrón	$m\mu$	$m\mu$	kilogramo	kg	kg
Angström	Å	Å	gramo	g	gm
microgramo	μg	μg	miligramo	mg	mg
gama	γ	γ	miliequivalente	mEq	mEq
hora	h	hr	Curie	c	c
minuto	m	Min	Milicurie	mc	mc
segundo	s	sec	Microcurie	μC	μC
			por ciento	%	%

Para evitar la confusión derivada de la notación decimal diferente según los países, se adopta el punto decimal y se suprime toda notación entre millares sustituyéndose por un espacio: 10 000 (no 10.000 ni 10,000) —0.90 (no 0,90).

Corrección de pruebas: Una de las pruebas de imprenta será remitida a los autores, quienes deberán devolverlas corregidas, dentro de los cuatro días subsiguientes a su recepción. Las modificaciones fundamentales en la corrección de las pruebas en desacuerdo con los originales, no serán tomadas en cuenta.

Pedido de apartados: Los autores que desen apartados podrán solicitarlos al devolver las pruebas. El costo correrá por su cuenta.

SUMARIO

Influence of Postural Changes on Ammonia Excretion by the Kidney in Ammonium Chloride Loading. — A. C. Taquini, J. D. Fermoso, P. Aramendía, I. de la Riva, B. N. Badano and M. F. Villamil ... 235

On the Integration of Respiratory Movements. — J. García Ramos .. 246

On the Integration of Respiratory Movements II. The Integration at Spinal Level. — J. García Ramos and E. López Mendoza 257

The Pressure in the Different Zones of the Pericardium. — J. L. Duomarco, C. E. Giambruno and A. Correa Durán 267

Functional Modifications of the Pacemaker and Conducting Systems of the Heart in Experimental Hyper- and Hypothyroidism. —H. Van Den Beld C., R. Douglas R. and J. Talesnik D. 273

Induction of Arginase Synthesis in an Auxotroph of neurospora Crassa. — Julio Cabello, Ruth Urbá, Victoria Prajoux and Carlos Basilio 286

Las Contracciones Isotónicas de los Músculos Estriados. — Rafael Rubio G. 298

La Distribución de la Tensión en el Músculo Durante el Reposo y la Contracción. — Rafael Rubio G. 306

The Effects of Estrogens on Experimental Atherosclerosis. — Manuel René Malinow 313

The Influence of the Neck Tourniquet on the Blood Antidiuretic Potency of Rats. — L. Barnafi, S. Vial and H. Croxatto 338

Mechanism of the Effect of Thyroxine on the Adrenal Gland. — Carmen Grado, Edith Dubrock and Jaime Talesnik 341

Índice, Autores 349

Materias 352